Peripheral neuropathic changes in pachyonychia congenita

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Abstract

We compared patterns of intraepidermal nerve fibers and mechanoreceptors from affected and unaffected plantar skin from patients with pachyonychia congenita (PC) and control subjects. Plantar biopsies from 10 genetically confirmed patients with PC (with a mutation in KRT6A) were performed at the ball of the foot (affected skin) and the arch (unaffected) and were compared to biopsies from corresponding locations in 10 control subjects. Tissue was processed to visualize intraepidermal nerve fibers (IENF) (PSP9.5), subsets of IENF (CGRP, substance P, tyrosine hydroxylase), myelinated nerve fibers (neurolamin H, NFH), blood vessels (CD31), Meissner corpuscles, and Merkel cells (MCs). Structures were quantified using stereology or validated quantification methods. We observed that PC-affected plantar skin had significantly lower sweat gland innervation (sweat gland nerve fiber density) and reduced numbers of Meissner corpuscles compared to PC-unaffected or anatomically matched control skin. In contrast, Merkel cell densities and blood vessel counts were higher in PC-affected skin compared to either control or PC-unaffected skin. There were no differences in myelinated nerve fiber densities, SP, or CGRP between the groups. Pressure pain thresholds in PC-affected skin were lower compared to PC-unaffected and anatomically matched control skin. Additionally, MC densities in callused plantar skin from healthy runners with callus and one subject with a nonpainful palmoplantar keratoderma (AQP5 mutation) were similar to PC-unaffected and control skin consistent with callus alone not being sufficient to increase MC number. These findings suggest that alterations in PC extend beyond keratinocytes and may provide strategies to study neuropathic pain in PC.

Keywords: Merkel cell, Intraepidermal nerve fiber, Sweat gland innervation, Neuropathic pain, Pachyonychia congenita

1. Introduction

Pachyonychia congenita (PC) is an autosomal dominant keratoderm caused by a mutation in any one of the keratin genes KRT6A, KRT6B, KRT6C, KRT11, or KRT17. Plantar hyperkeratosis, thickened nails, oral plaques, and intense pain (in the feet) are the distinct characteristics of PC.14 Pressure on affected areas of soles is painful, resulting in patients limiting their activity. Shaving PC-associated callus only partially alleviates pain, and “over shaving” is also paradoxically painful. Little is known regarding the origin, nature, or underlying mechanisms of pain in these patients.

In the present study, we investigated the histopathology and distribution of cutaneous nerve fibers, subsets of epidermal nerve fibers, meissner corpuscles, myelinated nerves, and blood vessels in patients with PC. Skin samples were obtained from affected thickened plantar skin and from an unaffected nearby area in PC subjects and from the corresponding areas in healthy control subjects.

2. Materials and methods

2.1. Patients

Ten subjects (50% female) with genetically confirmed PC (KRT6A mutations) and 10 control subjects (50% female) underwent 3-mm plantar skin punches at the level of the tarsal/metatarsal joint and at the arch through the International Pachyonychia Congenita Research Registry (WIRB #20040468). The tarsal/metatarsal region contained affected skin in PC subjects, whereas the arch biopsy contained unaffected epidermis. Control subjects underwent biopsies at both locations to address potential anatomic differences. An additional 3 subjects served as callus controls and had biopsies obtained from the ball of the foot where there was callus. Two of these subjects had thick callus due to running, whereas the third subject had a nonpainful palmoplantar keratoderma (AQP5 mutation). Biopsies were obtained after local anesthesia with subcutaneous lidocaine with 2% epinephrine.

No PC subject had peripheral neuropathy or potential cause for peripheral neuropathy, such as diabetes, B12 deficiency, hypothyroidism, or alcohol use greater than 2 drinks per day. Control subjects were part of a Johns Hopkins-approved protocol and had no known risk factors or symptoms for peripheral neuropathy and had normal peripheral nerve examinations, including vibration threshold, pin sensation, reflexes, light touch, and Von Frey filament assessment (controls detected using a 0.07-g filament).
Six PC subjects with KRT6A mutations and 10 control subjects underwent assessment for pressure pain thresholds in the sole (ball and arch).

2.2. Laboratory procedures

Skin samples were processed using an established protocol. They were fixed overnight in Zamboni fixative, rinsed in phosphate-buffered saline, and then cryoprotected. Three to four 50-μm-thick sections were immunohistochemically stained for each marker using a conventional avidin–biotin complex technique with SG peroxidase substrate (Vector Laboratories, Burlingame, CA) as the chromogen. Double- or triple-labeled immunofluorescence was used to reveal patterns of co-expression/distribution. Antibody details are provided (Table 1) and determined to show no cross-reactivity. For triple labeling, 3 primary antibodies raised in different species were detected with species-specific secondary antibodies conjugated with Cy2, Cy3, or Cy5 and viewed on a Nikon or Zeiss J1 microscope fitted with selective optical filters.

PGP9.5 was used as a panaxonal marker. Large myelinated fibers, including those forming Meissner corpuscles, were identified by their immunoreactivity to neurofilament H. Merkel cells were labeled using cytokeratin 20 (CK20). Peptide-containing unmyelinated sensory fibers were identified by immunoreactivity to CGRP. Blood vessels were labeled using antibodies against CD31.

2.3. Quantification of intraepidermal nerve fibers, dermal innervation, and vasculature

Intraepidermal nerve fiber density (IENFD) quantification was performed using established counting rules and expressed as fibers/mm. A blinded technician performed all quantification and determined to show no cross-reactivity. For triple labeling, 3 primary antibodies raised in different species were detected with species-specific secondary antibodies conjugated with Cy2, Cy3, or Cy5 and viewed on a Nikon or Zeiss J1 microscope fitted with selective optical filters.

As per convention, the length of epidermis at the stratum corneum to calculate linear densities and the value was measured using Stereo Investigator (MicroBrightField, Williston, VT). The average of 3 randomly selected sections per biopsy was expressed as the number per mm. The density of nerve fibers innervating sweat glands was quantified using an unbiased stereologic approach, as described previously.

Blood vessel density in dermal papilla was determined by counting the number of vessels that intersected with a line parallel to the skin surface, 50 μm above the bottom of the rete ridges (expressed as vessels per mm).

Conical fluorescence microscopy was used to assess colocalization and patterns of expression of 2 or more markers. Since neurofilament H (NFH)-, CGRP-, and substance P (SP)-labeled nerve fibers are located mainly in the subepidermal plexus where innervation can vary, we applied a technique of unbiased sampling. Briefly, immunohistochemistry involving multiple markers was performed under a uniform protocol, and all images were captured at the same exposure condition and were processed with Zeiss Zen 2012 software for measuring signal intensity in a 200 × 100-μm box (20,000 μm²), at random around every third optical field through the whole length of each evaluated section. Comparisons of numbers of neuronal profiles were made between control and PC groups and within the PC group between affected and unaffected skin areas.

2.4. Sensory testing

Pressure pain thresholds were measured using an Algometer (SBmedic Hornby, Sweden) with a 1-cm-diameter circulator probe. Force was applied at a constant rate until the subject experienced pain. Two trials at the ball and arch were averaged at each location.

2.5. Statistics

Statistical analysis was performed using Prism 5 (GraphPad Software, La Jolla, CA). Subjects’ ages are presented as mean ± SD, whereas group averages for other measurements are presented as mean ± SEM. Comparisons between PC-affected and PC-unaffected samples were performed by paired Wilcoxon nonparametric tests, and Mann–Whitney rank sum analyses were used for control and PC comparisons. Pachyonychia congenita–affected skin was compared to anatomically matched biopsies (ball of the foot) from healthy controls, whereas PC-unaffected skin from the arch was compared to arch samples from control subjects. We adjusted for multiple comparisons (Table 2) using the false discovery rate method.

3. Results

3.1. Study subjects

The PC subjects ranged in age range from 19 to 71 years (49.0 ± 15.4 years), whereas controls ranged from 28 to 62 years (46.6 ± 10.7 years). Each group had 5 men and 5 women. All PC subjects

### Table 1

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List of antibodies used in this study including: name, target structure, source, catalogue no., host, type, dilution, and detection method. Secondary antibodies conjugated with Cy2, Cy3, or Cy5 were obtained from Jackson Immunoresearch (West Grove, PA).

CGRP, calcitonin gene-related peptide; CGRP, cytokinin 20; NFH, neurofilament heavy; SP, substance P; TH, tyrosine hydroxylase.
possessed KRT6A mutations. Nine of 10 PC subjects reported pain that affected their daily life and limited walking. Foot pain had prompted 7 of 10 to purchase special shoes or make modifications to their shoes. Standing, walking, or heat made pain worse in 8 of 10 subjects. Resting (7 of 10) and cold temperatures (5 of 10) were identified as relieving factors. Sweating changes were reported in 7 of 10 subjects, with 6 reporting increased sweating and 1 reporting reduced sweating, although locations were not specified.

To assess the role of thick callus, we studied 3 additional “callus control” subjects. Two were runners who had thick plantar callus and the third was a patient with a nonpainful palmoplantar keratoderma (AQP5 mutation) characterized by thick plantar callus. The density of Merkel cells (MCs) and the IENFD from these subjects were similar to those of other control subjects. Additionally, callus control group (2.1 ± 0.7), and callus control group (2.1 ± 0.4), though not significantly (Fig. 2C, D). Moreover, the nerve fibers in the dermal papilla of the PC-affected skin had thinned and frequently fragmented appearances (Fig. 2A). In biopsies taken from callus control subjects, mean IENFD was intermediate between that of control ball of foot skin and affected PC skin. However, these differences did not reach statistical significance. We also examined cutaneous innervation with nerve fiber subsets expressing CGRP, a known marker for nociceptive peptidergic C fibers (Fig. 3A–C, fibers stained in white). In control biopsies, CGRP-labeled fibers were concentrated in the subepidermal plexus area, whereas they were rarely seen in the epidermis, in contrast to findings in rodents. Quantification of fluorescent intensity revealed no significant differences between PC-affected, PC-unaffected, and control skin (Fig. 3D). Nerve fiber subsets labeled by SP and tyrosine hydroxylase were present almost exclusively in the dermis and were similar between PC-affected, PC-unaffected, or control skin.

PC-affected skin (Fig. 4A) had significantly lower sweat gland nerve fiber density (SGNFD) when compared to either PC-unaffected skin (Fig. 4B) (P < 0.05) or control skin (Fig. 4C) from the ball of the foot (the same anatomic location as PC-affected skin, P < 0.05) (Fig. 4D).

3.4. Sensory and autonomic unmyelinated nerve fiber innervation

PGP9.5-derived IENFD from control plantar biopsies (both the arch and the ball of the sole) were substantially lower than what was previously observed in hairy skin at the distal leg,29,40 a finding consistent with the previously described rostral-caudal pattern of decreasing innervation that has been described. Pachyonychia congenita–affected skin (Fig. 2A) had significantly lower IENFD (1.2 ± 0.4) than PC-unaffected skin (Fig. 2B) (4.2 ± 3.2 fibers/mm, P < 0.05), and IENFD from ball of foot biopsies was notably lower among the PC-affected compared to anatomically matched control biopsies (2.8 ± 0.7), and callus control group (2.1 ± 0.4), though not significantly (Fig. 2C, D). Moreover, the nerve fibers in the dermal papilla of the PC-affected skin had thinned and frequently fragmented appearances (Fig. 2A). In biopsies taken from callus control subjects, mean IENFD was intermediate between that of control ball of foot skin and affected PC skin. However, these differences did not reach statistical significance. We also examined cutaneous innervation with nerve fiber subsets expressing CGRP, a known marker for nociceptive peptidergic C fibers (Fig. 3A–C, fibers stained in white). In control biopsies, CGRP-labeled fibers were concentrated in the subepidermal plexus area, whereas they were rarely seen in the epidermis, in contrast to findings in rodents. Quantification of fluorescent intensity revealed no significant differences between PC-affected, PC-unaffected, and control skin (Fig. 3D). Nerve fiber subsets labeled by SP and tyrosine hydroxylase were present almost exclusively in the dermis and were similar between PC-affected, PC-unaffected, or control skin.

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3.3. Control and unaffected PC cutaneous measures

Unaffected PC epidermis (from the arch) was indistinguishable from control arch epidermis in appearance and in all structures assessed.
green labeling) were abundant in the upper dermis and particularly dense in the area close to the dermal–epidermal junction, where they appeared to innervate Merkel cells or form Meissner corpuscles (see below). This pattern is notably different from leg hairy skin, where we observed much fewer NFH\(^1\) fibers, consistent with the description that the density of large myelinated fibers has a proximal to distal increase.\(^4^3\) Quantification of NFH\(^1\) axons in the dermis was similar in PC-affected, PC-unaffected, and control skin (Fig. 3E).

### 3.6. Mechanoreceptors: Merkel cells, Meissner corpuscles

Rodent and human studies have shown that MCs are abundantly expressed in footpads and palmar skin, particularly in touch domes.\(^3^5,4^2\) This fact, combined with the sensitivity to pressure that patients with PC report, prompted us to examine MC and Meissner corpuscles. Merkel cells appear as oval structures approximately 10\(\mu\)m in their longest diameter (Figs. 5 and 6) and were distributed, often in clusters, at the dermal–epidermal junction of the rete ridges (Fig. 5A–C). Meissner corpuscles are...
easily identified by their characteristic coiled appearance and are located primarily in the dermal papillae. Merkel cells were more numerous in PC-affected epidermis compared to PC-unaffected (\(P < 0.001\)) or anatomically matched control skin (\(P < 0.01\), Fig. 5D). They were often present in clusters in PC-affected skin compared to PC-unaffected or control biopsies from the ball of the foot. In contrast, MC densities in biopsies taken from callus control skin were similar to those of other control samples. Unlike MC, Meissner corpuscles were present at very low density, often only 1 or 2 corpuscles per 4 skin sections. The density of Meissner corpuscles was reduced in affected PC skin (\(0.037 \pm 0.018\) corpuscles/mm, \(P < 0.05\)) or anatomically matched control skin (\(0.33 \pm 0.10\) corpuscles/mm, \(P < 0.05\)) or callus control subjects (\(0.25 \pm 0.09\), \(P < 0.05\)).

Double immunohistochemistry with CK20 and NFH (Fig. 6A) revealed that most MCs are in close proximity to a dense network of NFH\(^+\) nerve terminals. Given that pain is the main symptom in PC, and that Meissner corpuscles were reduced in affected PC skin (0.037 ± 0.018 corpuscles/mm) compared to PC-unaffected (0.16 ± 0.047 corpuscles/mm, \(P < 0.05\)) or anatomically matched control skin (0.33 ± 0.10 corpuscles/mm, \(P < 0.05\)) or callus control subjects (0.25 ± 0.09, \(P < 0.05\)). Double immunohistochemistry with CK20 and NFH (Fig. 6A) revealed that most MCs are in close proximity to a dense network of NFH\(^+\) nerve terminals. Given that pain is the main symptom in PC, and that Meissner corpuscles were reduced in affected PC skin compared to PC-unaffected or control biopsies from the ball of the foot. In contrast, MC densities in biopsies taken from callus control skin were similar to those of other control samples. Unlike MC, Meissner corpuscles were present at very low density, often only 1 or 2 corpuscles per 4 skin sections. The density of Meissner corpuscles was reduced in affected PC skin (\(0.037 \pm 0.018\) corpuscles/mm, \(P < 0.05\)) or anatomically matched control skin (\(0.33 \pm 0.10\) corpuscles/mm, \(P < 0.05\)) or callus control subjects (\(0.25 \pm 0.09\), \(P < 0.05\)).

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3.7. Blood vessels

Patients with PC identify petechial-like lesions in affected skin that are particularly painful. The composition of these structures is unknown, although the shared growth mechanisms of axons and vessels prompted us to evaluate the pattern and distribution of blood vessels. Blood vessels were abundant in dermal papillae of both PC subjects (Fig. 7A, B) and controls (Fig. 7C), although vessels from PC-affected skin (Fig. 7A) followed a more tortuous path consistent with the engorged varicose vein appearance described in ultrasound studies. The altered topology of PC-affected samples resulted in vessels having a strikingly elongated pattern that paralleled the deep dermal papillae (Figs. 1D and 7A). The number of blood vessels in PC-affected biopsies (18.6 ± 0.6) was increased compared to PC-unaffected samples (11.9 ± 0.8), healthy control samples (12.8 ± 1.1), or callus control (12.8 ± 0.7) (Fig. 7D).

3.8. Sensory testing

Subjects with PC reported discomfort with significantly less pressure in affected skin than nonaffected skin (96 ± 86 kPa vs 444 ± 142 kPa, \(P < 0.01\)). Among control subjects, there was no...
difference between the ball and arch of the foot (542 ± 143 kPa vs 466 ± 188 kPa) (Fig. 8).

4. Discussion

We compared innervation patterns of nerve fibers and mechanoreceptors in plantar glabrous skin from PC-affected (painful) skin, PC-unaffected (nonpainful) skin, and anatomically matched biopsies from healthy controls. There were no differences between PC-unaffected and control subject skin in any of the markers studied. Painful PC-affected skin demonstrated multiple alterations, including increased density of MC and blood vessels and reduced numbers of Meissner corpuscles and densities of small unmyelinated nerve fibers (SGNFD) compared to nonpainful skin.

Figure 4. Reduced sweat gland innervation in PC-affected skin. Representative image of sweat glands immunostained for PGP9.5 (green) to show nerve fibers, with co-labeling for CD31 (red) and DAPI (blue) to show associated structures. Sweat gland innervation is lower in PC-affected skin (A) as compared to PC-unaffected skin (B) and corresponding skin of healthy controls (C). (D) Quantitative analysis. Data are shown as mean ± SEM, n = 10 for each point. *# indicates gland follicles. PC, pachyonychia congenita; SGNFD, sweat gland nerve fiber density; *P < 0.05, **P < 0.01. Bars = 50 μm.

Figure 5. Increased Merkel cell (MC) density in PC-affected skin. (A) PC-affected, (B) PC-unaffected, (C) normal control. (D) MC density by group. Immunohistochemical staining for CK20 (red) was performed to visualize MC. The MCs appear as spherical red structures (white arrows). The MCs are located at the epidermal–dermal interface and usually in clusters. Data in (D) are shown as mean ± SEM, n = 3 for callus control, n = 10 for all other points. CK20, cytokeratin 20; der, dermis; epi, epidermis; PC, pachyonychia congenita. ***P < 0.001, **P < 0.01. Bar = 100 μm.
PC or anatomically matched control epidermis. Taken together, these findings suggest that PC has an unappreciated neuropathological component that may provide insight into the neuropathic pain these patients experience.

Pachyonychia congenita–affected skin architecture is markedly altered compared to control or unaffected-PC epidermis. The stratum corneum is dramatically thickened, and papillary crypts are deep, extending into the stratum corneum resulting in an undulated dermal–epidermal junction. We chose epidermal length at the stratum granulosum and not at the undulating dermal–epidermal junction based on the belief that skin surface is most salient to sensation.

Pachyonychia congenita is perceived as a dermatological condition, yet foot pain is the largest complaint. “Burning,” “stinging,” and “electric-shock sensations” are common descriptors, and a PC symptom survey concluded that PC has a neuropathic pain component. Several mechanisms could explain the histological alterations we observed: (1) The unmyelinated and thinly myelinated nerve fiber loss reflects a focal neuropathic process; (2) increased Merkel cell numbers results in pathological mechanical hypersensitivity; (3) alterations in PC-affected keratinocytes lead to generation of pain; and (4) the abnormal architecture of PC-affected epidermis results in altered cutaneous biomechanics, thereby producing pain.

Reductions in IENFD and SG innervation are accepted measures of peripheral neuropathy and have been described in a number of neuropathic conditions, including diabetes, HIV infection, chemotherapy, and inherited neuropathy among others. These changes have been described in hairy skin and to a lesser extent in glabrous fingertip skin. Here, we observed a reduction in SGNFD in PC-affected epidermis and

**Figure 6.** Characterization of Merkel cell (MC) innervation. Representative confocal images for CK20, NFH, and CGRP immunohistochemistry are shown. The (A) series shows MC and NFH staining demonstrating that the majority of MCs (red) are in close proximity to NFH axon (green); (A') depicts CK20 (red) immunohistochemistry, (A''') depicts NFH (green) immunohistochemistry, and (A'''') is overlay of CK20 and CGRP. The (B) series shows MC and CGRP immunohistochemistry; (B') depicts MCs by CK20 (red) immunohistochemistry, (B'') depicts results of CGRP (blue) immunohistochemistry, (B''') depicts NFH (green) immunohistochemistry, (B''') is the overlay of MC (red), CGRP (blue), and NFH (green) immunohistochemistry. Majority of MCs appear as pink (red + blue), indicating that MCs express CGRP. (C) Percentage of MC that express CGRP by group. Data are shown as mean ± SEM, n = 3 for callus control, n = 10 for all other points. (D) High magnification imaging shows that MCs are apposed by bouton-like nerve terminals, expressing both NFH and CGRP (white arrowheads), CGRP alone (white arrows), or single labeled by NFH (*). CK20, cytokeratin 20; NFH, neurofilament H. Bar: (A and B) 5 μm, (D) 2.5 μm.
a trend toward reduced IENFD. Together these results suggest that PC has a neuropathic component that might explain the pain experienced by patients with PC. Indeed, the discomfort that patients with PC describe is similar to small fiber neuropathy. The pain in both is worse at night and aggravated by walking or standing. The SGNFD reduction in PC-affected skin suggests an underappreciated dysautonomia that may contribute to dryness and skin cracking that can occur in the soles of patients with PC. How small fiber damage leads to pain is not fully understood though the irritable nociceptor hypothesis, whereby damaged nerve fibers have increased excitability and ectopic activity would account for the cardinal features of PC. For example, nerve injury–induced increases in the abundance or sensitivity of pronociceptive ion channels (eg, transient receptor potential, voltage-gated sodium, piezo) or pronociceptive receptors (eg, neurotrophin receptors, G-protein-coupled receptors) or downregulation of hyperpolarizing potassium channels or inhibitory G-protein-coupled receptors in nociceptive neurons might account for such changes. Quantitative examinations of these potential contributors to PC pain are warranted.

The sensitivity to pressure in PC-affected skin prompted us to assess the pattern and density of mechanoreceptors. There was a dramatic reduction in the number of Meissner corpuscles in PC-affected vs unaffected skin that was even more striking when comparing PC-affected to anatomically matched control skin. In contrast, MC density was dramatically increased in PC-affected vs PC-unaffected or control skin. Merkel cells are excitatory cells capable of Ca\textsuperscript{2+} action potentials and MC–neurite complexes function as slowly adapting type I mechanoreceptors. They are believed to tune mammalian touch receptors, possess high spatial resolution with selective sensitivity to edges, curves and corners, and encode surface features. This process involves Piezo2 mechanically gated ion channels expressed in both the MC and the associated myelinated nerve fibers, and Piezo2 is required for MC mechanotransduction. Furthermore, Piezo2 knockdown in MC reduces capsaicin-induced mechanical allosthenia.
whereas BDNF release from MC shapes the physiology of associated SAI mechanoreceptive fibers, suggesting that MC may play an important role in mechanical allodynia and potentially explaining how increased MC densities contribute to pain in PC-affected skin. Consistent with this hypothesis, we observed a sensory correlate of significantly reduced pressure pain thresholds in PC-affected skin vs. PC-unaffected or control skin.

Many MCs were closely juxtaposed to NFH+ nerve fibers. We were surprised, however, that a subset of these NFH+ fibers and MC also expressed CGRP. Merkel cells are understood to be innervated by SAI nerve fibers and the proximity and potential functional interaction between MC and CGRP+ nerve has not been previously appreciated, to our knowledge. Since CGRP-innervated MC could represent an example of phenotypic switching, we assessed the fraction of MC juxtaposed to CGRP+ fibers and the fraction of CGRP+ MC. There were no differences in either parameter across the study groups. Yet, because of the overall increased MC number, the absolute number of MC in contact with CGRP+ fibers and of CGRP+ MCs were elevated in PC-affected vs unaffected skin.

The observation that only PC-affected skin is painful suggests that keratinocytes could themselves be responsible for this pain. Indeed, keratinocyte stimulation is sufficient to evoke acute nociception-related responses. Furthermore, certain protein families involved in neuronal nociception (transient receptor potential channels) have also been shown to perform functions related to epidermal homeostasis and barrier function.27,56 Most notable among these is TRPV3, a channel in which gain-of-function mutations lead to Olmsted syndrome, a painful palmoplantar keratoderm that shares many features with PC.33,54 Additionally, specialized touch dome keratinocytes regulate both MC abundance and innervation by associated SAI mechanoreceptor fibers. It is possible that an alteration in keratin expression and the keratin filament cytoskeleton changes keratinocyte-neuronal interactions and contributes to neuropathic pain. For example, keratinocytes from PC-affected skin might express higher levels of neurotrophic factors, leading to increased MC numbers or sensory neuron excitability that ultimately contributes to pain. Nerve growth factor (NGF) produces neurotrophic changes in skin and contributes to neuropathic pain, whereas keratinocyte overexpression of NT3 increases touch dome–associated MC numbers. Abundance and survival of MC is also dependent on the transcription factor Atoh1. RNA profiling experiments comparing PC-affected and PC-unaffected tissue from patients with several different keratin mutations identified differential expression of genes involved in epidermal development and conification but not in NGF, NT3, or Atoh1 transcript levels. Alternatively, keratin expression in neurons and/or MC might be altered in PC. Merkel cells, for example, are known to express keratins 14 and 20,17 and this might contribute to MC abundance in PC-affected skin.

The altered architecture in PC-affected skin could also contribute to pain hypersensitivity. The elongated rete ridges, for example, might lead to increased stimulation of axonal and/or keratinocyte pain pathways under normal mechanical loading conditions. Merkel cells have cytoplasmic protrusions and hemidesmosomes that physically link them to surrounding epithelial cells, whereas collagen fibers link the external capsule of Meissner corpuscles to both the lamellar cells and the epidermis. The altered architecture in PC-affected epidermis could influence signaling from these structures. Similarly, callus in PC-affected skin might contribute to the pathological and pathophysiological changes associated with this condition. However, in our study, callused plantar skin from 2 runners and from a subject with nonpainful palmoplantar keratoderma was neuroanatomically similar to the other control subjects, with only a slight trend toward decreased IENFD and no changes in MC or vascular density.

Patients with PC often identify petechial-like spots on their soles as being particularly painful. A decrease in these structures was associated with less pain and reduced callous in an open-label trial. Recognizing that nerve and blood vessels share developmental pathways, we assessed the pattern of blood vessel growth in PC epidermis. Pachyonychia congenita–affected skin possessed elongated, highly branched, and more numerous vessels compared to PC-unaffected or control skin.

Finally, it is possible that several of these mechanisms are involved. For example, in the setting of reduced IENFD, any associated changes in the function of remaining dermal nociceptor terminals might lead to central sensitization, which would in turn manifest more strongly by input from the increased number of MC and their associated mechanoreceptors. Concomitantly, phenotypic switching might occur, with mechanoreceptors conveying nociceptive stimuli. This could be enhanced by the altered epidermal/dermal architecture in PC-affected skin, causing increased MC stimulation. Regardless, it is clear that the skin contains numerous sensory structures that function in concert and that many of these structures are altered in PC-affected skin and may contribute to neuropathic pain.

Although we adjusted for multiple comparisons, a larger sample is needed to assess whether similar patterns are present in other forms of PC due to mutations in KRT6B, KRT6C, KRT16, or KRT17 or in other palmoplantar keratodermas.

The observation that PC-affected skin contains alterations in peripheral nerve structures broadens the perception of PC from a dermatological condition to a multisystem disorder with neuropathic pain as a prominent feature. These observations open the door to novel treatment approaches and suggest that neuropathic pain treatments may have a role in PC.

Conflict of interest statement

M. J. Caterina is an inventor on a patent on the use of products related to TRPV1, which is licensed through UCSF and Merck, and has consulted for Hydra Biosciences, which produces TRP channel–related products. These conflicts are being managed by the Johns Hopkins Office on Policy Coordination.

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References


