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Pathophysiology of pachyonychia congenita-associated palmoplantar keratoderma:

New insight into skin epithelial homeostasis and avenues for treatment

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Bulleted statements:

What's already known about this topic?

- Pachyonychia congenita is a rare genodermatosis caused by mutations in *KRT6A*, *KRT6B*, *KRT6C, KRT16*, *KRT17,* which are normally expressed in skin appendages and induced following injury.
- Individuals with PC present with multiple clinical symptoms that usually include thickened and dystrophic nails, palmoplantar keratoderma (PPK), glandular cysts, and oral leukokeratosis.
- The study of PC pathophysiology is made challenging because of its low incidence and high complexity. There is no cure or effective treatment for PC.

What does this study add?

- This text reviews recent progress made when studying the pathophysiology of palmoplantar keratoderma (PPK) associated with pachyonychia congenita (PC).
- This recent progress points to new possibilities for devising effective therapeutics that may complement current palliative strategies.

Abstract

Pachyonychia congenita (PC), a rare genodermatosis, primarily affects ectoderm-derived epithelial appendages and typically includes oral leukokeratosis, nail dystrophy, and very painful palmoplantar keratoderma (PPK). PC dramatically impacts quality of life though it does not affect lifespan. PC can arise from mutations in any of the wound repair-associated keratin genes *KRT6A*, *KRT6B*, *KRT6C*, *KRT16*, or *KRT17*. There is no cure for this condition, and current treatment options for PC symptoms are limited and palliative in nature. This review focuses on recent progress made towards understanding the heterogeneity and pathophysiology of PPK lesions, the most prevalent and debilitating of all PC symptoms. In particular, the study of the *Krt16* null mouse, which spontaneously develops footpad lesions that mimic several aspects of PC-associated PPK, revealed three main stages of PPK lesion progression. Ahead of lesion onset, keratinocytes in the palmoplantar (footpad) skin of *Krt16* null mice exhibit specific defects in differentiation. At the time of PPK onset, there is elevated oxidative stress and hypoactive Keap1-Nrf2 signaling. During active PPK, there is a profound defect in the ability of the epidermis to maintain or return to normal homeostasis. The progress made suggests new avenues to explore for the

treatment of PC-based PPK and deepens our understanding of the mechanisms controlling skin tissue homeostasis.

Pachyonychia congenita (PC; OMIM #1672000 and 167210) is a rare genodermatosis with a collection of symptoms primarily affecting ectoderm-derived appendages including oral leukokeratosis, nail dystrophies, sebaceous cysts, natal teeth, and palmoplantar keratoderma (PPK). While PC does not impact lifespan, it dramatically impacts quality of life for affected individuals. For instance, individuals with PC experience severe plantar pain from PPK lesions daily, often making everyday tasks difficult. There is currently no known cure or effective therapeutics for the treatment of PC [1].

PC can arise from autosomal dominant mutations in any of five keratin genes including *KRT6A*, *KRT6B*, *KRT6C*, *KRT16*, or *KRT17* [1-5]. These keratins are normally expressed in epithelial appendages and are otherwise robustly inducible, e.g., after injury or exposure to environmental stresses, together accounting in part for the clinical presentation of this disorder. Most PC-causing mutations are missense alleles, with occasional mall insertions or deletions in the keratin coding sequence. Until recently, two majors types of PC, type 1 (Jadahsson-Lewandowsky [6]) and type 2 (Jackson-Lawler [7]), were recognized based on their prevalent clinical features. Nowadays, five subtypes of PC are recognized based on genetic etiology - for example, PC caused by a *KRT6A* mutation corresponds to the PC-K6a subtype. Owing in part to the heterogeneity in the clinical presentation of PC (even among patients with very similar alleles), a definitive diagnosis can only be ascertained through sequencing of these keratin genes [1,8,9].

The Pachyonychia Congenita Project

The PC Project is a USA public charity that was founded in 2003 and has evolved into a life-changing resource for both individuals with PC and for clinicians and researchers interested in this condition. This organization connects individuals with PC and their families to others with this condition, and to clinicians, translational and basic science researchers. The PC project provides assistance to individuals with PC to attend support meetings and qualify for genetic testing. Further, the PC Project is home to the International PC Research Registry (IPCRR), which gathers extremely valuable data from questionnaires, photos, and notes on genetically-confirmed PC cases. As of January 2019, the IPCRR includes 864 genetically confirmed cases of PC in 49 countries. This PC registry has evolved into a transformative resource for patients, clinicians and researchers working together towards understanding this disorder and developing effective therapeutics. Finally, the PC Project plays a lead role in fostering basic and clinical research on PC. More information about the PC Project and how to get involved can be found on the publicly-available website www.pachyonychia.org.

Asserting a focus on palmoplantar keratoderma

While individuals with PC present with many symptoms of significance, PPK is highly penetrant and reportedly most debilitating [1] (Fig. 1A). Virtually all individuals with PC (> 90% [1]) present with PPK lesions restricted primarily to pressure points in palmar and/or plantar epidermis and consisting of dramatic epidermal thickening and hyperkeratosis [3-5,10]. PPK lesions are debilitating in part because of the extreme pain associated with them [11-13]. Interestingly, these lesions do not display signs of keratinocyte fragility and/or lysis. The latter represents a predominant element in epidermolysis bullosa simplex (EBS), a genetically-determined skin blistering condition caused by mutations in either *KRT14* or *KRT5* [14-17]. Keratinocyte fragility also is a dominant pathophysiological feature in epidermolytic PPK, which is often caused by mutations in *KRT9* [18], the major differentiation-specific keratin in volar epidermis [19,20]. The greater complexity of keratin gene expression in volar epidermis likely contributes to maintain keratinocyte structural integrity in spite of mutations in individual genes such as *KRT6A-C*, *KRT16* and *KRT17*. This said, the pathophysiology of PC-associated PPK is only partially understood at present, reflecting significant limitations related to the low incidence of this orphan disease and the severe pain associated with these lesions [21,22]. Accordingly, there is no effective treatment for PC-based PPK. The current standard of care for PPK consists of routine removal of calluses followed by treatment with moisturizers [23] (see below for details). A deeper understanding of the pathophysiology of PPK might spearhead the development of effective therapeutics for individuals with PC and also inform researchers, clinicians and drug developers on other genetic and clinical subtypes of PPK (Fig. 1B). This text focuses on recent progress made in deciphering the pathophysiology of PC-associated PPK lesions.

A short primer on the nomenclature of PC-associated keratin genes

The original catalog of human keratin proteins devised by Moll et al. [24] already recognized the existence of K6 as a type II, and of K16 and K17 as type I, keratins. However, the true diversity of keratin genes and proteins was underestimated until the advent of whole genome sequencing efforts [25], which necessitated a revision of the Moll nomenclature [26]. Per the internationally accepted nomenclature, human genes are designated using capital lettering (e.g., *KRT16*) and mouse genes are designated using normal size lettering (e.g., *Krt16*). The multiplicity of K6 sequences was originally uncovered in the human [27]. Currently, we know of two functional genes in the mouse, *Krt6a* and *Krt6b* [28], and three functional K6 genes in the human, *KRT6A*, *KRT6B*, and *KRT6C* [2,29]. By contrast, a single gene codes for each of K16 [30,31] and K17 proteins [32,33] in the human and mouse genomes. The high degree of conservation known to apply to orthologous keratin genes in the mouse and human, in terms of sequence features and regulation,

applies to the PC-associated keratin genes [29,31,32]. This information is relevant to discussing the utilization of transgenic mouse models to study keratin mutation-based human conditions such as PC.

Lessons learned from transgenic mouse models

As there are no *in vitro* human cell culture models that can be used to investigate the cellular and molecular mechanisms underlying PPK pathophysiology or screen potential therapeutics, researchers have relied on the use of transgenic mouse models (summarized in Table 1) to study PC and PPK [34]. Among the models available, the *Krt16* null mouse strain is the only one that spontaneously develops footpad skin lesions mimicking PC-associated PPK lesions. Characterization of *Krt16* null mice has revealed three phases in PPK, each with a somewhat unique molecular signature: pre-PPK, PPK onset, and active PPK (Fig. 2).

In 2-weeks old *Krt16* null mice, corresponding to the "pre-PPK stage", footpad skin keratinocytes exhibit defects in selective aspects of terminal differentiation. At this early time point there are minimal alterations to the skin tissue histology but, already, a dramatic loss of keratin 9 (*Krt9*/K9) expression has occurred, which then persists throughout lesion progression [35]. *Krt9* occurs exclusively in differentiating keratinocytes of volar skin and represents a predominant marker gene in this setting [19,20,36]. In contrast to *Krt9*, several differentiation markers appear to be upregulated in *Krt16* null footpad skin, potentially as a compensatory mechanism [35]. While this partial defect in terminal differentiation is currently unexplained [35], it occurs independent and ahead of the oxidative stress phenotype observed at a later stage of progression of PPK-like lesions in this mouse model [35,37].

In one-month old *Krt16* null mice, corresponding to "onset stage" of PPK-like lesions, footpad skin epidermis displays several features of oxidative stress, including decreased levels of the master cell antioxidant glutathione and decreased expression of glutathione synthesis genes compared to *WT* controls. Keap1-Nrf2 signaling, a central regulator of the cellular antioxidant response, is markedly attenuated at that time while Nrf2 itself, a transcription factor, is upregulated though ineffective in *Krt16* null footpad skin (likely reflecting an attempt to restore redox homeostasis). While difficult to ascertain given restricted access to plantar skin biopsies from PC patients, there is evidence of reduced Nrf2 activity in PC-PPK lesions of individuals with *KRT16* mutations [37].

In two-months old *Krt16* null mice, corresponding to an "active stage" of PPK, there is a profound defect in the ability of footpad skin to maintain or return to normal tissue homeostasis. By this time all *Krt16* null mice have spontaneously developed PPK-like lesions on their paws, and while these lesions preferentially arise in areas exposed to the substratum and thus experiencing mechanical stress, they are not associated with keratinocyte fragility [38,39]. At a molecular level, the *Krt16* null footpad lesions

exhibit a gross misregulation of many Danger Associated Molecular Patterns (DAMPs) and barrier homeostasis genes, which mimics human PC-based PPK lesions [39].

Lessons learned from computational endeavors

Along with targeted molecular analyses, computational analysis of genomic data sets has also provided significant insight into the pathophysiology of PC-based PPK. Systems genetics has been used to explore the role of K16 in regulating the skin's response to stress. Re-analyzing a powerful systems genetics data set that related the risk of developing skin tumors to the regulation of skin inflammation and barrier function [40] revealed a tight link between *Krt16*, skin barrier genes, and innate immunity effectors including DAMPs [39,41]. In this data set, moreover, *Krt16* expression is significantly correlated with expression of barrier homeostasis and inflammation genes in tail skin, both at baseline and in response to TPA, which acts as a chemical irritant [41]. The discovery that *Krt16* belongs to a network of barrier homeostasis genes pointed to a role for *Krt16* in calibrating the skin's response to barrier-compromising stresses [39], which converged nicely with the phenotype of PPK-like lesions exhibited by *Krt16* null mice. These efforts lent strong support to the notion that a better understanding of how K16 calibrates the skin's stress response could be applicable to PC as *KRT16* expression is often elevated in PC-based PPK lesions.

The availability of genome-wide surveys of gene expression from both *Krt16* null footpad skin lesions [35] and PPK lesions from individuals with PC [22] has provided an excellent opportunity to further test the strengths and limitations of the *Krt16* null mouse as a valid model for PC-based PPK. Merging the human PPK datasets with the murine *Krt16* null footpad lesions dataset, based upon human-mouse orthologous gene pairings, enabled multiple computational analyses [35]. Pairwise comparisons of global transcriptional changes in *Krt16* null footpad lesions and individual PC cases (3 *KRT6* cases, 3 *KRT16* cases) generated statistically significant positive correlation values in all cases (Fig. 3A-B). Additionally, pairwise comparisons of global transcriptional changes further highlight the high degree of heterogeneity between individual cases involving different keratin mutations, and between cases with the same mutated keratin allele (Fig. 3C). Altogether these comparisons provided a strong case that lesional *Krt16* null mouse footpad skin mimics PC-associated PPK lesions at a global gene expression level, reinforcing and extending the notion that the *Krt16* null mouse is an appropriate model for the study of pathogenesis of PC-associated PPK lesions.

A role for keratin imbalances and genetic background in the pathophysiology of PPK

Since the presentation of PC symptoms varies greatly between individuals even with similar or the same mutated keratin allele [1,42-44], there likely is a role for genetic background and gene modifiers in

the pathophysiology of this condition. Remarkably, similar alleles in *KRT16* (N125S and R127C) can elicit a presentation of focal non-epidermolytic PPK vs. full-blown PC [44-46] (Fig. 1C), suggesting that the consequences associated with alterations in *KRT16* are subject to modifier gene(s) effects. Consistent with this notion, despite the immunological differences between mice and humans, several phenotypic aspects of *Krt16* null mice including the PPK-like lesions are modestly impacted by genetic strain background [47]. Interestingly, select phenotypic traits in *Krt17* null mice [48] and *Krt6a/Krt6b* double-null mice [49] also exhibit a dependence on genetic background.

In addition to genetic background, imbalances in keratin expression also appear likely to play a significant role in pathophysiology of PC-based PPK. For example, the differentiation-specific keratins *KRT2* and *KRT9* are both decreased in *Krt16* null footpad lesions and human PC-based PPK [22,35,50]. Of note, mice that are double-null for the differentiation-specific *Krt2* and *Krt10* develop a keratoderma-like phenotype on footpad skin [51] while mice null for *Krt9* develop an epidermolytic PPK that closely resembles the corresponding human disorder [36]. Aside from these (and other) alterations (see [35] and [22]), the expression of *KRT6A*, *KRT6B*, *KRT16*, and/or *KRT17* (including the mutated alleles) is dramatically increased in PC-based PPK, as expected given the stress- and wound-sensitive regulation of these genes[22]. Given the knowledge that K6, K16 and K17 proteins have pleiotropic and context-dependent properties [35,37,39,52-56], such alterations in keratin protein levels and balance among them are poised to have a striking impact on the development and evolution of PPK lesions.

Pathophysiological unknowns in PPK and other clinical features of PC

While all clinical manifestations associated with PC are worth a deep investigation, two of them stand out as remaining particularly intriguing at a cellular and molecular level. One is the occurrence of individual or multiple cysts (steatocystoma multiplex; see OMIM entries #184500 and #184510) in PC patients, which are benign fluid filled cysts believed to originate from sebaceous glands and which can occur all over the body and arise preferentially in individuals with mutations in *KRT17* [57-59]. These cysts often require surgical drainage or removal as their rupture and/or inflammation pose a risk for infection and can be painful for patients [1]. Another intriguing manifestation is natal teeth, which refers to presence of teeth in newborns and is also preferentially associated with mutations in *KRT17* [1,59]. Natal teeth are soft, friable and prone to caries, and are usually lost within the first few months of life [1,60-63]. Of note, *Krt17* is expressed at a very early stage of the development of ectodermal appendages, including the tooth [32]. Recent studies have shown that genetic variants in the PC-associated keratin genes increase susceptibility to tooth decay [64]. There is currently no model to study the cystic skin lesions and phenomenon of natal teeth associated with PC.

Limitations of past and current therapeutic strategies for PC

       PC-associated PPK has been treated with a combination of keratolytics, pain medication, orthotics, and mechanical removal of calluses [23,65]. While the keratolytics salicylic acid and urea soften calluses, they cannot control the significant overgrowth associated with most cases of PC-PKK. Pain medication and custom orthotics can partially alleviate discomfort, but do not treat the underlying PPK. Routine mechanical removal of calluses by filing, grinding, or cutting has been the most satisfying treatment for individuals with PC [23]. Significant efforts are currently under way to develop new and effective therapeutics for the management of these lesions. Two distinct strategies are highlighted here. The first strategy involves the development of short interfering RNAs (siRNAs) that specifically target mutant keratin alleles and reduce their expression. It has shown some promise in a trial of the siRNA TD101, which targets the *KRT6A* N171K allele, albeit in a single patient [21,66,67]. In its current form, this approach suffers from the limitation that delivery of such nucleic acid-based therapeutics requires intradermal injections that cause intense pain to the patient. The generation of self-delivery siRNAs for mutant keratin alleles improves the uptake of siRNAs by keratinocytes [68] but does not improve penetration through the stratum corneum. Accordingly, a method to deliver siRNA-based therapeutics that involves topical application of therapeutic agents is sorely needed [69].

       The second strategy to treat PC-based PPK consists of drug-based interventions aimed at reducing mutant keratin gene expression. The mTOR inhibitor rapamycin/sirolimus suppresses K6a expression and, when taken orally, improves PC symptoms [70]. However, severe side-effects associated with systemic rapamycin treatment prevent it from being a viable long-term treatment for PC. Recently, topical sirolimus treatment of two K6a patients improved PC-PPK without the toxicity of systemic treatment [71] but requires additional studies to confirm the safety and efficacy of this treatment. Oral retinoids successfully reduced callus thickness in some PC individuals, but like rapamycin, adverse side effects including increased pain prevent oral retinoids from being a viable long-term treatment for PC [72]. Statins can also downregulate *KRT6A* expression [73], but so far only oral rosuvastatin has been shown to be effective in a single case of K6a based PC [74]. Finally, injections of botulinum toxin (Botox) into plantar calluses improved plantar blistering and pain associated with PC-PPK lesions [75], but injections are costly and must be performed under anesthesia. While each of these drug-based interventions provide some relief, none of them in present form provide viable long-term treatment strategies for PC-based PPK.

Opportunities for novel therapies

       A promising opportunity to complement ongoing efforts to develop effective therapeutics for PC-based PPK would be to target stress response pathways and/or pathways capable of promoting the restoration of normal epidermal differentiation. In *Krt16* null mice, topical treatment with the small

natural molecule sulforaphane (SF), which activates Nrf2 signaling by modifying Keap1 [76], can prevent

Year	Mouse model	Genetic modification	Main phenotype(s)	References
1996	*Krt6aΔ21P*	Deletion of 52 amino acids (residues 125-176) between head and 1A helix domain	Intraepidermal blistering	82
1999	*Krt6a transgenic*	Truncation deleting the 2B region of the central rod domain	Lethal blister or alopecia	83
1999	*Krt6a*	Replacement of E2 by HK1-tag	Hyperkeratosis and late-	83

PPK-like lesions in male mice [37]. Addition of the ER-β agonist Diarylpropionitrile (DPN) to the SF treatment regimen is necessary for successful activation of Nrf2 [77] and prevention of PPK-like lesions in female mice. SF is available in pure form or as part of broccoli sprout extract [78], can be safely delivered topically, and has shown therapeutic promise in the treatment of EBS arising from mutations in either keratins K5 or K14 [79,80]. The sexual dimorphism in response to SF treatment in mice is a reminder that sex-based differences is an important consideration when developing therapeutics for any disease [81]. Whether there is a sexual dimorphism in the setting of PC remains an open question.

Another strategy worth considering is to normalize terminal differentiation in volar skin. In male *Krt16* null mice treated with SF prior to lesion onset, restoration of Nrf2 activity coincided with induction of *Krt9* expression [35]. Additional efforts should be focused on testing this specific strategy. In the end, the prospect of combining treatment modalities that act to prevent and/or treat active lesions represent an attractive prospect for the treatment of a condition featuring the complexity of PC-associated PPK.

Future directions

Pachyonychia congenita (PC) is a monogenic skin disease with a complex, polygenic presentation. Despite the plethora of challenges that arise in studying this disease, the use of transgenic mouse models and of computational biology has been invaluable and provided novel insight into the pathophysiology of PC-based PPK, one of the most debilitating symptoms for individuals with PC. The study of PPK pathophysiology not only paves the way for researchers to devise therapeutics to treat PC, but also provides an opportunity to better understand the mechanisms that control skin tissue homeostasis.

Acknowledgements

	transgenic		onset alopecia	
2000	*Krt6a/Krt6b*^{-/-}	Deletion of *Krt6a* and *Krt6b* locus	Oral lesions	49 84
2000	*Krt6a*^{-/-}	Deletion of *Krt6a* locus	Delay of reepithelialization after wounding	85
2002	*Krt17*^{-/-}	Deletion of *Krt17* locus	Age and strain dependent alopecia	48
2005	*Krt6a/Krt6b*^{-/-}*; Krt17*^{-/-}	Deletion of *Krt6a*, *Krt6b*, and *Krt17* locus	Severe cell lysis in nail bed epithelium	86
2008	*Krt75 knock-in*	Point mutation of codon N158 (corresponding to mutation N171 in PC case)	Defects in hair shaft, nail fragility	87
2011	*KRT6A N171K Humanized skin*	Bioengineered skin equivalents derived from PC individuals with N171K mutation engrafted onto immunodeficient mice	Acanthosis and epidermal blistering	88
2012	*Krt16*^{-/-}	Deletion of *Krt16* locus	Oral lesions, footpad lesions resembling human PPK	38,47

Table 1: Mouse models with phenotypes that are potentially relevant to pachyonychia congenita (PC).

Figure Legends

Figure 1: Palmoplantar keratoderma, a genetically heterogeneous disorder. (A) Photograph of PC-based PPK lesions from an individual with a *KRT16* L124R mutation. Source: Pachyonychia Congenita Project (www.pachyonychia.org). (B) Table summarizing the diversity of genes which, when mutated, can elicit a PPK clinical presentation. Various clinical subtypes of PPK are accounted for. Additional references for table are 18,89-111. (C) Schematic of select mutations in keratin 16 (K16) protein that are causative for PC, focal non-epidermolytic PPK (FNEPPK), or both PC and FNEPPK. K16 exhibits the tripartite domain structure shared by all IF proteins, with a N-terminal "head" domain, central α-helical "rod" domain, and C-terminal "tail" domain. The central rod domain is comprised of heptad repeat-containing α-helical coils (1A, 1B, Coil 2) separated by non-heptad repeat linkers (L1 and L12). Many attributes of

the central rod domain (see red bars) are highly conserved and represent a signature element among IF proteins. Representative mutations that are causative for FNEPPK are in blue text, mutations causative for PC are in gold text, and mutations that are causative for both FNEPPK and PC are in purple text.

Figure 2: Development of PPK-like lesions in *Krt16* null footpad skin proceeds in three stages: Pre-PPK (at 2 weeks of age), Onset of PPK (at 4 weeks), and Active-PPK (at 8 weeks). (A) Representative histology of *Krt16* null footpad skin at 2-, 4-, and 8-weeks of age. At 2 weeks, the epidermis shows a normal thickness and overall architecture but, upon closer inspection, alterations including the abnormal appearance of the granular layer, crowding of basal keratinocytes, and a decreased nuclear aspect ratio of basal keratinocytes can be seen. At 4 weeks, prior to macroscopic appearance of lesions, mild epidermal thickening is observed. By 8 weeks, there is dramatic thickening of the living epidermis and the stratum corneum, infiltration of immune cells, and limited suprabasal cell lysis. Dotted line is epidermal/dermal junction. Derm = dermis. Epi = epidermis, SC = stratum corneum. Scale bar = 100 μm. Images acquired using a Zeiss microscope with Apotome attachment and processed using Zen 2.3 software. (B) Summary of key molecular changes that occur at 2-, 4-, and 8-weeks of age in *Krt16* null footpad skin. (C) Potential therapeutic interventions for each stage of lesion development (see refs. 35,37,39,77). SF = sulforaphane. DPN = Diarylpropionitrile.

Figure 3: Correlation of transcriptional changes between *Krt16* null footpad lesions and human PC-based PPK lesions. (A-B) Correlation coefficients (R) calculated from pairwise comparisons of microarray data from *Krt16* null footpad lesions [35] and human PC-based PPK lesions [22] resulting from mutations in *KRT6* (A) or *KRT16* (B). (C) Correlation coefficients calculated from pairwise comparisons between individual cases of PC-based PPK with either *KRT6* or *KRT16* mutations. A R value of -1 would convey perfect negative correlation, whereas a R value of 1 conveys perfect correlation between samples. P values for each correlation are denoted in purple text underneath the corresponding R values. Figure adapted from Zieman et al. (2019) [35].

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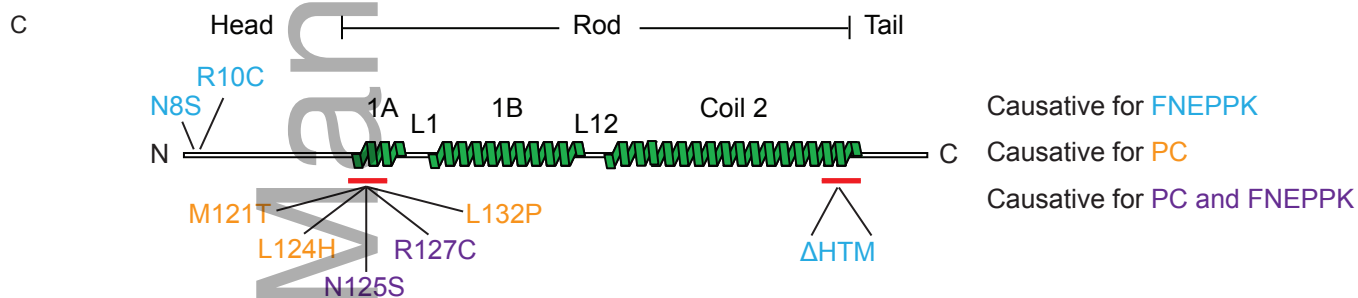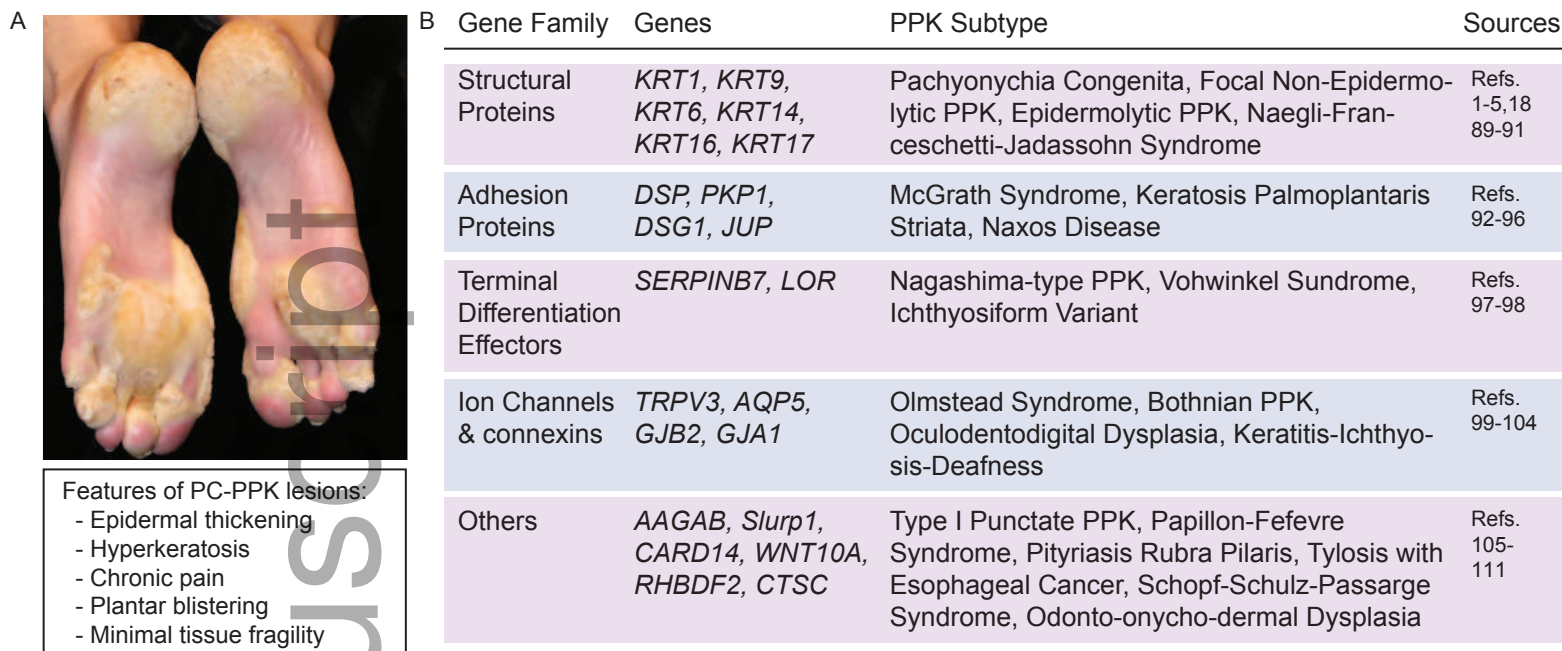
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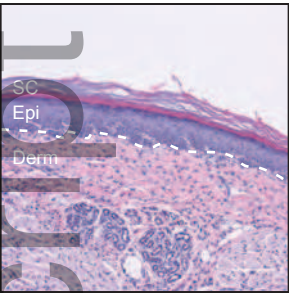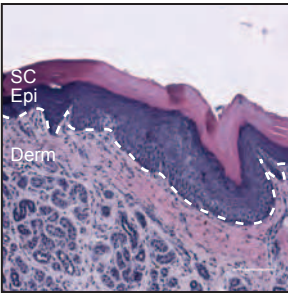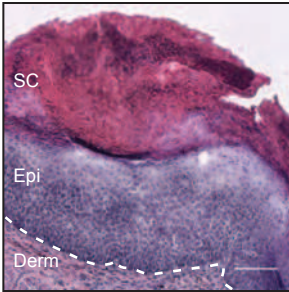
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A

Features of PC-PPK lesions:
- Epidermal thickening
- Hyperkeratosis
- Chronic pain
- Plantar blistering
- Minimal tissue fragility

B

Gene Family	Genes	PPK Subtype	Sources
Structural Proteins	*KRT1, KRT9, KRT6, KRT14, KRT16, KRT17*	Pachyonychia Congenita, Focal Non-Epidermolytic PPK, Epidermolytic PPK, Naegli-Franceschetti-Jadassohn Syndrome	Refs. 1-5,18 89-91
Adhesion Proteins	*DSP, PKP1, DSG1, JUP*	McGrath Syndrome, Keratosis Palmoplantaris Striata, Naxos Disease	Refs. 92-96
Terminal Differentiation Effectors	*SERPINB7, LOR*	Nagashima-type PPK, Vohwinkel Sundrome, Ichthyosiform Variant	Refs. 97-98
Ion Channels & connexins	*TRPV3, AQP5, GJB2, GJA1*	Olmstead Syndrome, Bothnian PPK, Oculodentodigital Dysplasia, Keratitis-Ichthyosis-Deafness	Refs. 99-104
Others	*AAGAB, Slurp1, CARD14, WNT10A, RHBDF2, CTSC*	Type I Punctate PPK, Papillon-Fefevre Syndrome, Pityriasis Rubra Pilaris, Tylosis with Esophageal Cancer, Schopf-Schulz-Passarge Syndrome, Odonto-onycho-dermal Dysplasia	Refs. 105-111

C

Head   Rod   Tail

R10C
N8S
1A L1 1B L12 Coil 2

N   C

Causative for FNEPPK
Causative for PC
Causative for PC and FNEPPK

M121T
L124H
N125S
R127C
L132P
ΔHTM

Zieman and Coulombe, Figure 1

Age of *Krt16* null mice

2 weeks Pre-PPK	4 weeks Onset of PPK	8 weeks Active PPK

A

B

| Altered Terminal Differentiation

Loss of *Krt9*/K9 expression | Oxidative stress

Hypoactive Keap1-Nrf2 signaling | Impaired barrier homeostasis

Inflammation

Elevated DAMPs |

C

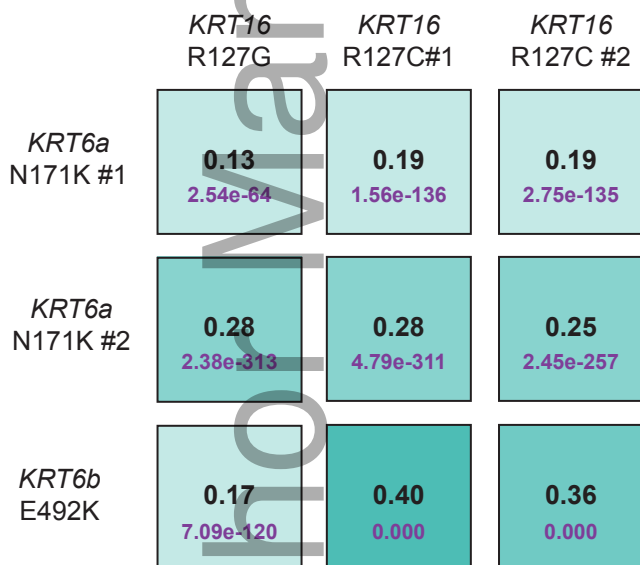| Restore *Krt9*/K9 expression via stimulation of terminal differentiation | Restore Nrf2 activity via topical application of SF (or SF+DPN) | Restore barrier homeostasis by targeting stress response path-ways |

Zieman and Coulombe, Figure 2

A Correlation between mouse *Krt16* null paw skin
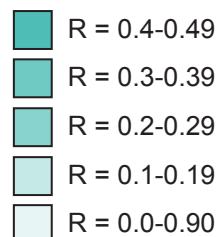   lesions and human PPK lesions (*KRT6* cases)

Krt16 null

| 0.081 | 0.19 | 0.18 | *KRT6a* N171K #1 |
| 6.41e-27 | 2.67e-135 | 2.80e-132 | |

| 0.13 | 0.29 | | *KRT6a* N171K #2 |
| 5.34e-63 | 0.000 | | |

| 0.19 | | | *KRT6b* E492K |
| 9.881e-137 | | | |
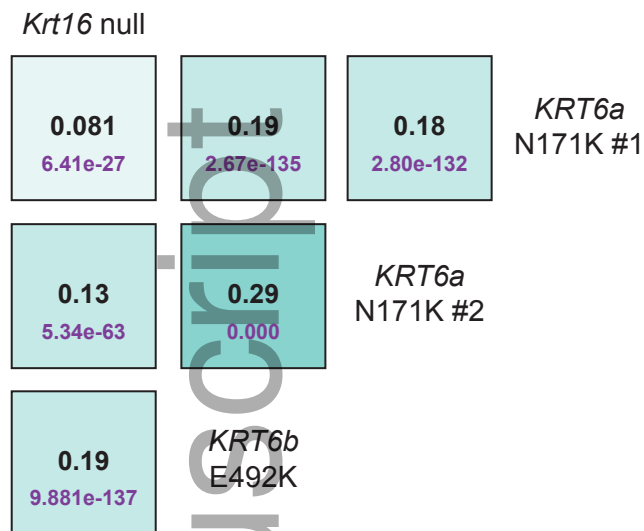
B Correlation between mouse *Krt16* null paw skin
   lesions and human PPK lesions (*KRT16* cases)

Krt16 null

| 0.062 | 0.29 | 0.30 | *KRT16* R127G |
| 6.72e-16 | 0.000 | 0.000 | |

| 0.12 | 0.48 | | *KRT16* R127C#1 |
| 3.09e-54 | 0.000 | | |

| 0.18 | | | *KRT16* R127C #2 |
| 2.43e-122 | | | |
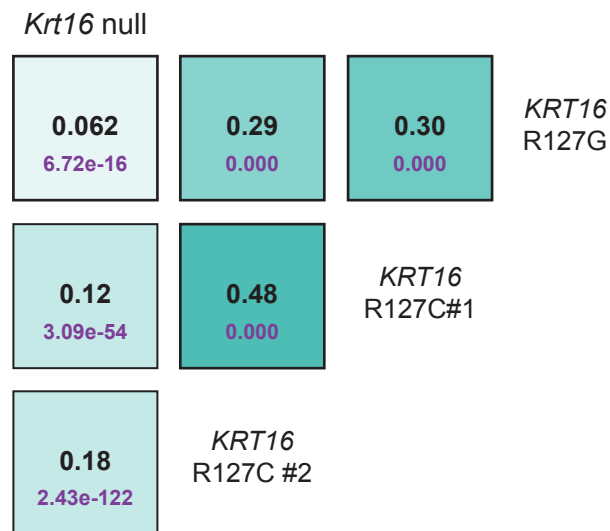
C Correlation between human *KRT6*- and
   KRT16-based PPK lesions

	KRT16 R127G	*KRT16* R127C#1	*KRT16* R127C #2
KRT6a N171K #1	0.13 / 2.54e-64	0.19 / 1.56e-136	0.19 / 2.75e-135
KRT6a N171K #2	0.28 / 2.38e-313	0.28 / 4.79e-311	0.25 / 2.45e-257
KRT6b E492K	0.17 / 7.09e-120	0.40 / 0.000	0.36 / 0.000

Guide

▉	R = 0.4-0.49
▉	R = 0.3-0.39
▉	R = 0.2-0.29
▉	R = 0.1-0.19
▢	R = 0.0-0.90

Highest Correlation:
K16R127C cases (R=0.48)

Lowest Correlation:
K6aN171K #1 and K16R127G (R=0.13)

Purple text: P values

Zieman and Coulombe, Figure 3