Pathophysiology of pachyonychia congenita-associated palmoplantar keratoderma: new insights into skin epithelial homeostasis and avenues for treatment*

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Accepted for publication
22 April 2019

Funding sources
These studies were supported by grant AR044232 issued to P.A.C. from the National Institute of Arthritis, Musculoskeletal and Skin Disease (NIAMS). A.G.Z. received support from grant T32 CA009110 from the National Cancer Institute.

Conflicts of interest
None declared.

*Plain language summary available online

DOI 10.1111/bjd.18033

Summary

Background 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 although 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.

Objectives This review focuses on recent progress made towards understanding the pathophysiology of PPK lesions, the most prevalent and debilitating of all PC symptoms.

Methods We reviewed the relevant literature with a particular focus on the Krt16 null mouse, which spontaneously develops footpad lesions that mimic several aspects of PC-associated PPK.

Results There are three main stages of progression of PPK-like lesions in Krt16 null mice. Ahead of lesion onset, keratinocytes in the palmoplantar (footpad) skin exhibit specific defects in terminal differentiation, including loss of Krt9 expression. At the time of PPK onset, there is elevated oxidative stress and hypoactive Keap1–Nrf2 signalling. During active PPK, there is a profound defect in the ability of the epidermis to maintain or return to normal homeostasis.

Conclusions 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.

What’s already known about this topic?

- Pachyonychia congenita (PC) is a rare genodermatosis caused by mutations in KRT6A, KRT6B, KRT6C, KRT16 and 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.
Pachyonychia congenita (PC; OMIM #1672000 and 167210) is a rare genodermatosis with a collection of symptoms primarily affecting ectoderm-derived appendages; it includes 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 small insertions or deletions in the keratin coding sequence. Until recently, two major types of PC – type 1 (Jadassohn–Lewandowsky6) and type 2 (Jackson–Lawler7) – were recognized based on their prevalent clinical features. Nowadays, five subtypes of PC are recognized based on genetic aetiology – 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 features), a definitive diagnosis can be ascertained only through sequencing of these keratin genes.1,8,9

The Pachyonychia Congenita Project

The Pachyonychia Congenita Project is a U.S. public charity that was founded in 2003 and has evolved into a life-changing resource for 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 Pachyonychia Congenita Project provides assistance to individuals with PC to attend support meetings and qualify for genetic testing. Further, the Pachyonychia Congenita Project is home to the International Pachyonychia Congenita 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 Pachyonychia Congenita Project plays a lead role in fostering basic and clinical research on PC. More information about the Pachyonychia Congenita 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 the most debilitating (Fig. 1a).1 Virtually all individuals with PC (> 90%) present with PPK lesions restricted primarily to pressure points in the palm and/or plantar epidermis and consisting of dramatic epidermal thickening and hyperkeratosis.3–5,16 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 is also a dominant pathophysiological feature in epidermolysis PPK, which is often caused by mutations in KRT9,18 the major differentiation-specific keratin in the volar epidermis.19,20 The greater complexity of keratin gene expression in the volar epidermis likely contributes to maintain keratinocyte structural integrity in spite of mutations in individual genes such as KRT6A-C, KRT16 and KRT17. That 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 (see below for details).23 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 pachyonychia congenita-associated keratin genes

The original catalogue of human keratin proteins devised by Moll et al.24 already recognized the existence of K6 as a type II
keratin, 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, which necessitated a revision of the Moll nomenclature. As per the internationally accepted nomenclature, human genes are designated using upper-case lettering (e.g. KRT6A), and mouse genes are designated using lower-case lettering (e.g. Krt6a). The multiplicity of K6 sequences was originally uncovered in the human. Currently, we know of two functional genes in the mouse, Krt6a and Krt6b, and three functional K6 genes in the human, KRT6A, KRT6B and KRT6C. In contrast, a single gene codes for each of K16 and K17 proteins 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. 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. 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-week-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 K9 (Krt9/K9) expression has occurred, which...
contrast to Krt16 upregulated in...satory mechanism. While this partial defect in terminal differ-
ence genes, which mimics human PC-based PPK lesions. In 2-month-old Krt16 null mice, corresponding to an ‘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 with WT controls. Keap1–Nrf2 signalling, 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 patients with PC, there is evidence of reduced Nrf2 activity in PC–PPK lesions of individuals with KRT16 mutations.

In 2-month-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 experience mechanical stress, they are not associated with keratinocyte fragility. At a molecular level, the Krt16 null footpad lesions exhibit a gross disorganization of many danger-associated molecular patterns (DAMPs) and barrier homeostasis genes, which mimics human PC-based PPK lesions.

**Lessons learned from computational endeavours**

Along with targeted molecular analyses, computational analysis of genomic datasets 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-analysing a powerful systems genetics dataset that related the risk of developing skin tumours to the regulation of skin inflammation and barrier function revealed a tight link between Krt16, skin barrier genes and innate immunity effectors including DAMPs. Moreover, in this dataset, Krt16 expression is significantly correlated with expression of barrier homeostasis and inflammation genes in tail skin, both at baseline and in response to 12-O-tetradecanoylphorbol-13-acetate (TPA), which acts as a chemical irritant. 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, 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 and PPK lesions from individuals with PC 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 on human–mouse orthologous gene pairings, enabled multiple computational analyses. Pairwise comparisons of global transcriptional changes in Krt16 null footpad

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

<table>
<thead>
<tr>
<th>Year</th>
<th>Mouse model</th>
<th>Genetic modification</th>
<th>Main phenotype(s)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996</td>
<td>Krt6a;A21P</td>
<td>Deletion of 52 amino acids (residues 125–176) between head and 1A helix domain</td>
<td>Intraepidermal blistering</td>
<td>82</td>
</tr>
<tr>
<td>1999</td>
<td>Krt6a transgenic</td>
<td>Truncation deleting the 2B region of the central rod domain</td>
<td>Lethal blister or alopecia</td>
<td>83</td>
</tr>
<tr>
<td>1999</td>
<td>Krt6a transgenic</td>
<td>Replacement of F2 by HK1-tag</td>
<td>Hyperkeratosis and late-onset alopecia</td>
<td>85</td>
</tr>
<tr>
<td>2000</td>
<td>Krt6a/Krt6b−/−</td>
<td>Deletion of Krt6a and Krt6b loci</td>
<td>Oral lesions</td>
<td>49, 84</td>
</tr>
<tr>
<td>2000</td>
<td>Krt6a−/−</td>
<td>Deletion of Krt6a loci</td>
<td>Delay of reepithelialization after wounding</td>
<td>85</td>
</tr>
<tr>
<td>2002</td>
<td>Krt17−/−</td>
<td>Deletion of Krt17 locus</td>
<td>Age- and strain-dependent alopecia</td>
<td>48</td>
</tr>
<tr>
<td>2005</td>
<td>Krt6a/Krt6b−/−; Krt17−/−</td>
<td>Deletion of Krt6a, Krt6b and Krt17 loci</td>
<td>Severe cell lysis in nail bed epithelium</td>
<td>86</td>
</tr>
<tr>
<td>2008</td>
<td>Krt75 knock-in</td>
<td>Point mutation of codon N158 (corresponding to mutation N171 in PC case)</td>
<td>Defects in hair shaft, nail fragility</td>
<td>87</td>
</tr>
<tr>
<td>2011</td>
<td>KRT6A N171K humanized skin</td>
<td>Bioengineered skin equivalents derived from individuals with PC with N171K mutation engrafted onto immunodeficient mice</td>
<td>Acanthosis and epidermal blistering</td>
<td>88</td>
</tr>
<tr>
<td>2012</td>
<td>Krt16−/−</td>
<td>Deletion of Krt16 locus</td>
<td>Oral lesions, footpad lesions resembling human PPK</td>
<td>38, 47</td>
</tr>
</tbody>
</table>

PPK, palmoplantar keratoderma
lesions and individual PC cases (three KRT6 cases, three 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 palmoplantar keratoderma

Because the presentation of PC symptoms varies greatly between individuals even with similar or the same mutated keratin allele, there is likely 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 nonepidermolytic PPK vs. full-blown PC (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. Interestingly, select phenotypic traits in Krt17 null mice and Krt6a/Krt6b double-null mice 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 the 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. Of note, mice that are double-null for the differentiation-specific

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**Fig 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, on 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 (Epi) and the stratum corneum (SC), infiltration of immune cells, and limited suprabasal cell lysis. The dotted line shows the epidermal–dermal junction. 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. DAMPs, damage-associated molecular pattern molecules; Derm, dermis; DPN, diarylpropionitrile; PPK, palmoplantar keratoderma; SF, sulforaphane.

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Krt2 and Krt10 develop a keratoderma-like phenotype on footpad skin while mice null for Krt9 develop an epidermolytic PPK that closely resembles the corresponding human disorder. Aside from these (and other) alterations, 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. Given the knowledge that K6, K16 and K17 proteins have pleiotropic and context-dependent properties, 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 palmoplantar keratoderma and other clinical features of pachyonychia congenita**

While all clinical manifestations associated with PC are worth a deep investigation, two stand out as remaining particularly intriguing at a cellular and molecular level. One is

**Fig 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 and human PC-based PPK lesions 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. An R-value of –1 would convey perfect negative correlation, whereas an 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. PC, pachyonychia congenita; PPK, palmoplantar keratoderma

Krt2 and Krt10 develop a keratoderma-like phenotype on footpad skin while mice null for Krt9 develop an epidermolytic PPK that closely resembles the corresponding human disorder. Aside from these (and other) alterations, 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. Given the knowledge that K6, K16 and K17 proteins have pleiotropic and context-dependent properties, 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 palmoplantar keratoderma and other clinical features of pachyonychia congenita**

While all clinical manifestations associated with PC are worth a deep investigation, two stand out as remaining particularly intriguing at a cellular and molecular level. One is
the occurrence of individual or multiple cysts (seostocystoma multiplex; see OMIM entries #184500 and #184510) in patients with PC, 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 KRT7.57–59 These cysts often require surgical drainage or removal as their rupture and/or inflammation pose a risk of 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 Nata 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.23 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 pachyonychia congenita

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–PPK. 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 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 mammalian target of rapamycin (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 patients with K6a-based disease improved PC–PPK without the toxicity of systemic treatment but requires additional studies to confirm the safety and efficacy of this treatment. Oral retinoids successfully reduced callus thickness in some individuals with PC, but like rapamycin, adverse side-effects including increased pain prevent oral retinoids from being a viable long-term treatment for PC.71 Statins can also downregulate KRT6A expression,23 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 anaesthesia.

While each of these drug-based interventions provides 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 signalling by modifying Keap1,76 can prevent PPK-like lesions in male mice.77 Addition of the ER-β agonist diarylpropionitrile to the SF treatment regimen is necessary for successful activation of Nrf277 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 are important considerations 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 vential 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

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 has 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.

Acknowledgments

The authors wish to thank members of the Coulombe laboratory for their support. This work was supported by grant AR044232 from the National Institutes of Health.

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