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Gene expression profiling in pachyonychia congenita skin

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ABSTRACT

Background: Pachyonychia congenita (PC) is a skin disorder resulting from mutations in keratin (K) proteins including K6a, K6b, K16, and K17. One of the major symptoms is painful plantar keratoderma. The pathogenic sequelae resulting from the keratin mutations remain unclear.
Objective: To better understand PC pathogenesis.
Methods: RNA profiling was performed on biopsies taken from PC-involved and uninvolved plantar skin of seven genotyped PC patients (two K6a, one K6b, three K16, and one K17) as well as from control volunteers. Protein profiling was generated from tape-stripping samples.
Results: A comparison of PC-involved skin biopsies to adjacent uninvolved plantar skin identified 112 differentially-expressed mRNAs common to patient groups harboring K6 (i.e., both K6a and K6b) and K16 mutations. Among these mRNAs, 25 encode structural proteins including keratins, small proline-rich and late cornified envelope proteins, 20 are related to metabolism and 16 encode proteases, peptidases, and their inhibitors including kallikrein-related peptidases (*KLKs*), and serine protease inhibitors (*SERPINs*). mRNAs were also identified to be differentially expressed only in K6 (81) or K16 (141) patient samples. Furthermore, 13 mRNAs were identified that may be involved in pain including nociception and neuropathy. Protein profiling, comparing three K6a plantar tape-stripping samples to non-PC controls, showed changes in the PC corneocytes similar, but not identical, to the mRNA analysis.
Conclusion: Many differentially-expressed genes identified in PC-involved skin encode components critical for skin barrier homeostasis including keratinocyte proliferation, differentiation, cornification, and desquamation. The profiling data provide a foundation for unraveling the pathogenesis of PC and identifying targets for developing effective PC therapeutics.

1. Introduction

Pachyonychia congenita (PC) is a rare keratinization disorder resulting from autosomal dominant-negative mutations in keratin genes that are expressed in epithelial tissues [1–3]. Symptoms include thickened dystrophic nails, palmoplantar keratoderma, leukokeratosis, and follicular hyperkeratosis [4–6]. The major patient complaint is painful plantar keratoderma (possibly due to fissuring, secondary infections, increased pressure on nerve endings, underlying blistering and/or cutaneous thromboses [7–10]), where intense pain negatively impacts quality of life and often necessitates ambulatory aides and pain medication.

Keratins heterodimerize and further associate to form intermediate filaments, which provide mechanical strength and resilience

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to epithelial cells and tissues [11,12]. Mutant keratins negatively impact skin structure and function, at least in part, by destabilizing the existing intermediate filament networks within cells [13–15], resulting in faulty intermediate filaments and cell fragility, which can lead to skin disorders including epidermolytic ichthyosis (also known as epidermolytic hyperkeratosis, caused by keratin (*KRT*) *1/KRT10* gene mutations) [16,17], epidermolysis bullosa simplex (*KRT5/KRT14*) [18,19], and PC (*KRT6A/6B/16/17*) [1,2,5]. The majority of PC mutations are heterozygous missense or small insertion/deletion mutations that disrupt cytoskeletal function, presumably by preventing proper protein/protein interactions [20,21]. In PC, this manifests as hyperplasia and hyperkeratosis in the subset of differentiated epithelial tissues in which mutant keratins including K6a, K6b, K16 and K17 are predominantly expressed [5], specifically in the palmoplantar epidermis, nail bed, oral mucosa, and the pilosebaceous unit. Surprisingly, *Krt16* knockout mice exhibit palmoplantar keratoderma (PPK)-like lesions, one of the hallmark features of PC [9,22], in the absence of keratin mutations, suggesting that the pathogenesis of PC is more complex than previously appreciated.

In this study, we performed gene expression microarray analysis of RNA extracted from PC patient biopsies to examine changes in mRNA expression in PC-involved versus uninvolved and non-PC plantar skin to better understand the pathogenesis of PC at the molecular level, including the intense idiopathic pain associated with plantar keratoderma. Proteomic profiling of stratum corneum supported the findings herein.

2. Materials and methods

2.1. Subjects

Seven patients from the International PC Research Registry (IPCRR) were identified by the Pachyonychia Congenita Project (www.pachyonychia.org) for evaluation in this study. Specific mutations in all seven IPCRR patients were previously identified, and the genotyping results were confirmed by a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory. The mutations of the participating PC patients are shown in Table 1. Using local anesthesia, a matched pair of 3 or 4 mm full-thickness plantar skin punch biopsies, one from PC-involved skin and the other from adjacent uninvolved skin (typically within 1–2 cm but as far as 5 cm), was collected from each patient listed in Table 1. The biopsy sites for one of the PC patients (IPCRR no. 1009, harbors a K16 R127C mutation) are shown in Fig. 1. Plantar biopsies from two healthy, non-PC volunteers (controls) were also collected from locations corresponding to the involved and uninvolved sites in PC patients. Biopsies were obtained using standard surgical techniques with patient consent under W-IRB no. 2004/0468/1057496.

2.2. RNA extraction and quantitative RT-PCR

Biopsied skin samples were collected and mechanically disrupted in a FASTprep24 (MPBio, Santa Ana, CA) with Lysing Matrix D as previously described [22,23]. Total RNA was isolated using the RNeasy Mini Plus kit (Qiagen), according to manufacturer's instructions. Total RNA (up to 1 μg) isolated from skin samples was reverse transcribed with random hexamer primers using the Superscript III First-Strand Synthesis System (Life Technologies, Grand Island, NY) following the manufacturer's instructions.

Table 1
IPCRR numbers and corresponding mutations of PC patients.

IPCRR no.	Mutation
8	K6a N171K
10	K6a N171K
661	K6b E472K
233	K16 R127G
1009	K16 R127C
1015	K16 R127C
394	K17 N92S

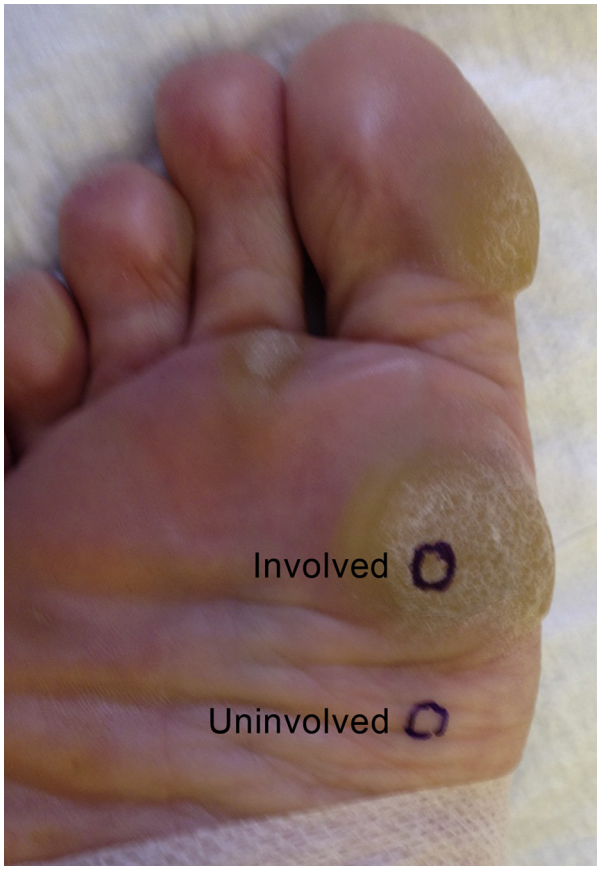

Fig. 1. Physical locations of plantar biopsy sites for one of the participating patients (K16-R127C mutation). Sites (involved and uninvolved) where biopsies were obtained (as described in Section 2) are circled. Similar biopsy pairs were collected from all PC participants and non-PC volunteers (controls).

Quantitative RT-PCR was performed on cDNAs (diluted 25-fold) from PC-involved and uninvolved biopsies of four patients (IPCRR no. 8, 233, 1009, and 1015). *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase), *KLK10, RGS20*, small proline-rich protein 1a (*SPRR1a*), *ADAM23*, *FABP5*, *RND3*, and *IFRD1* inventoried TaqMan gene expression assays were obtained from Applied Biosystems (ABI, Foster City, CA). Samples were analyzed using the ABI 7500 Fast Real-Time PCR System under standard conditions. The data were analyzed with the Applied Biosystem's Sequence Detection software (version 1.4) and reported as relative quantitation using *GAPDH* mRNA as the reference gene. All data points reported for individual patients are the mean of three replicate assays and changes in mRNA expression levels in PC-involved versus uninvolved are displayed as mean ± standard deviation ($n = 4$).

2.3. RNA profiling

RNA profiling was performed on RNA samples from all 7 patients listed in Table 1 as described in Supplemental materials and methods. Briefly, total RNA (100 to 200 μl of 50 ng/μl per sample) was processed following Agilent's (Santa Clara, CA) Two-Color Microarray-Based Gene Expression Analysis Protocol (Version 5.7).