



Pachyonychia Congenita Project

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We hope that making available the relevant information on Pachyonychia Congenita will be a means of furthering research to find effective therapies and a cure for PC.

Mutations in a Keratin 6 Isomer (K6c) Cause a Type of Focal Palmoplantar Keratoderma

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Twenty years have elapsed since keratin mutations were linked to cutaneous genodermatoses, and we now know that they cause 40 different genetic disorders. In this issue, Wilson *et al.* have identified *KRT6C* mutations in patients with focal palmoplantar keratoderma (FPPK), but debate concerning overlapping phenotypes between FPPK and pachyonychia congenita (PC) will continue because only one family has nail involvement. Furthermore, screening of control DNA samples identified 3 in 335 individuals (1%) who had a mutation (K6c p.Asn172del), but the phenotype was not ascertained. However, this raises the question as to whether individuals with sensitive feet bear specific *KRT6C* mutations and whether a general population screen should be considered.

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Keratin genodermatoses: 20 years down the road

It has been two decades since it was established that truncation of small specific regions of a keratin protein would severely affect the ability of keratin filaments to function normally. Although this affected only one of four genetic alleles involved in basic filament assembly and only 25% of the total protein content of the filament bore the small alteration, it was sufficient to cause filament collapse in cultured keratinocytes (Albers and Fuchs, 1987). A few years later, experiments with transgenic mice provided the first definitive link between keratin structural alterations and cutaneous genodermatoses (Vassar *et al.*, 1991). Single point mutations in specific keratin genes were then found to be causal in patients with two hereditary skin diseases: epidermolysis bullosa simplex, caused by mutations in K5 or K14 (Coulombe *et al.*, 1991), and epidermolytic hyperkeratosis, caused by mutations in K1 or K10 (Rothnagel *et al.*, 1992). Thus, single heterozygous point mutations in one allele were

sufficient to exert a dominant negative effect over the normal allele in the majority of cases, providing an explanation for the autosomal dominant nature of most keratin-based genodermatoses (Irvine and McLean, 1999).

Further research established the existence of more than 350 unique keratin mutations in 21 keratin genes that were the cause of 40 genodermatoses. Many keratin mutations were unique, but trends appeared that established hot spots for mutation in the genes that coincided with sensitive regions of the protein structure. Thus, alterations in the proximal 1A helical amino acid residues (helix initiation peptide) and the distal 2B helical residues (helix termination peptide) accounted for the majority (75%) of the known mutations. Within these regions, however, the sensitivity of specific amino acid residues varied, and mutation of adjacent residues could lead to significant differences in disease severity (Liovic *et al.*, 2004). In addition, evidence from family studies indicated that identical mutations could have different phenotypic

presentations in members of the same family, arguing for modulation of the phenotype by an individual's genetic background.

How many keratin genes?

It is well established that keratin intermediate filament proteins are essential components of the cytoskeleton of all mammalian epithelial cells. They form two large multigene families at separate chromosomal loci, with all type II keratin genes and a single type I gene (encoding K18) located at 12q11–q13 and the remaining type I keratin genes at 17q11–q21. Keratins are obligate heteropolymers, with filament assembly requiring a combination of type I and type II proteins. They were originally defined by their biochemical properties (Moll *et al.*, 1982; Bowden *et al.*, 1987)—the 11 type I keratins (K9–K19) were smaller (40–65 kDa) and more acidic (pI 4.5–6.0) than the 8 (K1–K8) type II keratins (50–70 kDa, pI 6.5–8.5). Recent bioinformatics based on the human genome project sequences has established that there are 54 functional keratin genes (abbreviated *KRT*): 31 epithelial keratins, 15 hair-specific keratins, and 8 inner root sheath keratins (Hesse *et al.*, 2004). Furthermore, a unified nomenclature was recently established for all known keratin intermediate filament genes and proteins (Schweizer *et al.*, 2006). This leaves 33 keratin genes in which mutations have not yet been identified and that are not yet associated with any genetic disorder.

Complications surrounding keratin 6 (K6)

Initially, two K6 proteins were identified (K6a and K6b), but evidence was presented for six isoforms of K6 (Takahashi *et al.*, 1995), each encoded by a separate gene (*KRT6a–6f*). This was followed by the discovery of another K6-sized protein in the companion layer (K6hf) and several specific keratins in the inner root sheath, termed K6irs. It was known from genodermatoses studies that mutations in K6a and K6b caused different

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phenotypic forms of pachyonychia congenita (PC) and that these were the probable partners of K16 and K17, respectively, but no evidence from genetic studies supported the existence of other K6 isoforms. The original six proposed isoforms were eventually reduced to three (K6a, K6b, and K6c) encoded by three functional isogenes (*KRT6A*, *KRT6B*, and *KRT6C*), based on sequence information from the human genome project and studies of the chromosomal locus (Rogers *et al.*, 2005). Furthermore, to avoid confusion, the other K6 proteins of the hair follicle were renamed (Schweizer *et al.*, 2006).

Mutations in the third K6 gene implicated as causal in focal palmoplantar keratoderma.

Mutations in *KRT6C*: another keratin gene nailed

In this issue, Wilson *et al.* implicate mutations in the third functional K6 gene (*KRT6C*) as causal in focal palmoplantar keratoderma (FPPK). These investigators have examined three FPPK families (two four-generation families and a two-generation family) and found a remarkably consistent phenotype with mainly plantar hyperkeratosis and blistering. There appears to be little if any palm involvement, but some minor nail changes were found in one family, suggesting a possible phenotypic overlap with PC. This nail disease has two major phenotypic variants: PC-1 is caused by mutations in *KRT6A* or *KRT16* (Bowden *et al.*, 1995; McLean *et al.*, 1995), whereas PC-2 is caused by mutations in *KRT6B* or *KRT17* (PC-2: McLean *et al.*, 1995; Smith *et al.*, 1998). Patients with focal nonepidermolytic palmoplantar keratoderma (PPK; FNEPPK: OMIM #613000) have been found to bear mutations in *KRT16* (Smith *et al.*, 2000), again representing a phenotypic overlap with PC-1. The FPPK phenotype also resembles another

palmoplantar disorder, hereditary painful callosities (HPC: OMIM #114140), in which some patients have lesions on the palms and others do not (Roth *et al.*, 1978; Baden *et al.*, 1984). Close examination of the clinical photographs in this issue and those previously published for HPC reveals a close similarity. In addition, preliminary data from two other HPC families with an identical phenotypic presentation confirm the ability of *KRT6C* mutations to cause pathology (Easter *et al.*, 2009.).

Palmoplantar keratoderma: a phenotypic mixed bag

Palmoplantar epidermis not only is thicker than the epidermis at other anatomical sites but also has a more complex pattern of keratin expression; it is an adaptive tissue capable of resisting mechanical trauma (Bowden *et al.*, 1987; Swensson *et al.*, 1998). Pathology of the skin at this location produces excessive thickening, blistering, and cell fragility, typical manifestations of a large group of genodermatoses collectively known as PPKs. The hyperkeratotic phenotype can be focal, striate, diffuse, or punctuate, and this can be the only pathology in a given family or part of a complex syndrome. The genetic cause has been elucidated for several of the PPKs; not only keratin gene mutations but also a wide range of other genes may be involved. Many of the phenotypic differences are minor or subtle, and isolating them into a group with a single genetic cause can be difficult. However, in the case of K6c mutations, the phenotype appears to be quite distinct. Perhaps more of these cases will come to light over the next few years, allowing a larger number of K6c mutations to be identified.

CONFLICT OF INTEREST

The author states no conflict of interest.

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Could Aging Human Skin Use a Connective Tissue Growth Factor Boost to Increase Collagen Content?

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The roles of connective tissue growth factor (CTGF) and transforming growth factor- β (TGF- β), both well-known collagen production stimulators, were examined in skin aging. Aged skin and fibroblasts exhibited a coordinate decrease in CTGF, TGF- β , and type I procollagen expression and content. CTGF knockdown and TGF- β blockade in normal dermal fibroblasts reduced procollagen expression, whereas overexpressing CTGF increased procollagen by a TGF- β /Smad signaling-dependent mechanism without involving Smad2/3.

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Background

Type I collagen is a major structural protein in human skin and, by mass, the most abundant protein in the human body. The association of age-dependent collagen loss with thinning and fragility of elderly skin has long been appreciated, yet the underlying mechanisms are not well understood. A recent study (Quan *et al.*, 2010, this issue) suggests that diminished expression of connective tissue growth factor (CTGF), together with diminished transforming growth factor (TGF)- β /Smad signaling, is responsible for this progressive loss of dermal collagen. CTGF is a secreted, matricellular protein, and, like that of other such proteins, its function is thought to be regulatory, not structural. CTGF is generally considered a pathogenic factor because of its many

reported disease associations (Leask *et al.*, 2009; Cicha *et al.*, 2009), potential macromolecular interactions, and complex gene regulation that also involves disease-associated processes and factors (Figure 1). The evolution of a purely pathogenic factor is unlikely without invoking an extreme selfish-gene concept, and the results of Quan *et al.*, which address CTGF function in normal young and aging skin, provide an alternative view of CTGF's physiological significance in tissue homeostasis.

CTGF in aging skin: new findings

By means of associative and mechanistic *in vivo* and *in vitro* studies, Quan *et al.* (2010) probe the importance of CTGF and TGF- β for collagen loss in aging skin. They report findings on expression, content, and

localization for CTGF, TGF- β , and type I procollagen- α 1, as well as CTGF's effects on TGF- β signaling. Using samples of young (21–30 years of age) and aged (80 or more years of age) but otherwise normal human skin, and laser-capture microdissected cells, the authors show that TGF- β and CTGF are normally expressed and produced in skin and skin fibroblasts. Comparing samples from young and aged subjects, Quan *et al.* found that in aged skin and fibroblasts, TGF- β , CTGF, and type I procollagen mRNA and protein levels were coordinately reduced. Using transfected normal human dermal fibroblasts, knockdown of CTGF was associated with decreased type I procollagen promoter activity (COL1A2), mRNA, and protein content, whereas overexpression of V5-tagged human CTGF increased the same readouts. Three approaches to block signaling due to endogenous TGF- β were used to assess molecular mechanisms by which CTGF modulates type I procollagen: (i) a specific TGF- β RI kinase inhibitor SB431542, (ii) Smad4 knockdown, and (iii) overexpression of inhibitory Smad7. All approaches completely blocked the CTGF-mediated increase in type I procollagen expression and production, indicating that TGF- β receptor and Smad signaling are required. However, no effects of CTGF knockdown or overexpression were observed on TGF- β -dependent Smad2/3 phosphorylation or Smad3 transcriptional activity.

On the basis of these findings, the authors conclude that endogenous production of both TGF- β and CTGF in human dermal fibroblasts normally acts to modulate type I procollagen expression in skin. They propose that this involves a TGF- β /Smad/CTGF axis that is operated by interdependent, yet distinct, mechanisms to regulate type I procollagen production. Decreased expression of TGF- β and CTGF by aged skin fibroblasts is proposed to underlie age-associated downregulation of the TGF- β /Smad/CTGF axis, thereby leading to reduced type I procollagen expression. These results suggest beneficial effects of CTGF and provide novel insight into the mechanisms of skin aging.

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