# Close Shave for a Keratin Disorder—K6hf Polymorphism Linked to Pseudofolliculitis Barbae

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An explosion of interest in genetic disorders of keratinization followed the discovery, in 1991, of the first keratin mutations in K5 and K14 causing the inherited skin blistering disorder epidermolysis bullosa simplex. The current tally is 19 keratin genes linked to human genetic disorders, as listed in Table I, including the report of a K6hf polymorphism by Hermelita Winter and colleagues in this issue of the JID. The key hallmark of all the keratin diseases is fragility of epithelial cells due to loss or weakening of the cytoplasmic keratin network (Irvine and McLean, 1999). Thus, the initial discoveries of keratin mutations in epithelial fragility syndromes demonstrated that the primary function of the keratin intermediate filament cytoskeleton is to provide epithelial cells with an internal scaffold to resist traumatic damage. Following on from this work, several keratin-associated proteins and other intermediate filament proteins were also linked to human tissue fragility diseases (Irvine and McLean, 1999). Since many of these involve phenotypes affecting the skin and its appendages, this has been a major growth area in dermatology over the past decade. The last few years, however, have seen a slowing down in the rate of discovery of genes being linked to new keratinizing disorders. This is in keeping with a general trend in human genetics in recent years with the advent of the Human Genome Project, where the rate of discovery of disease genes is still high but has reached a plateau as most of the obvious and more common genetic disorders have been cracked. This trend is exemplified by the rate of gene discovery in the genodermatoses (Irvine and McLean, 2003). Thus, there have not been many new additions to the keratin disease "hit list" in recent times.

In recent years, the incredible complexity of keratin gene expression within the hair follicle has come to light with major contributions coming from Jürgen Schweizer, Mike Rogers, Lutz Langbein and colleagues in Heidelberg. Of the many new keratins uncovered by the human genome project, most appear to be expressed in highly specific cell populations within the "soft" outer epithelial layers of the hair follicle that surround the hair shaft, for example the four new type II keratins of the inner root sheath, K6irs1-4 (Langbein *et al*, 2003) and their presumed type I keratin partners (Bawden *et al*, 2001). Another of the newer keratins, K6hf, is specifically expressed in the companion layer of scalp hairs (Winter *et al*, 1998; Wang *et al*, 2003), as shown in Fig 1. The latter is a single layer of cells between the outer root sheath and the inner root sheath. Antibodies and *in situ* hybridization probes directed against these new epithelial keratins reveal the exquisite compartmentalization within the hair follicle, making this the most complicated epithelial structure in humans, second only to the closely related avian feather follicles in terms of complex tissue architecture. These reagents represent powerful new tools for examining hair follicle gene expression and differentiation in a variety of experimental contexts and identifying the origin of hair follicle-derived tumors. The promoters of these genes will also allow specific expression of transgenes or ectopic gene therapy constructs within specific regions of the follicle in the future.

So what of genetic diseases of the hair follicle keratins? Although K5 and K14 are expressed in the outer root sheath of the follicle, which is continuous with the basal layer of the epidermis, patients with even the most severe forms of EBS with mutations in these genes do not have hair abnormalities. Therefore, the outer root sheath keratinocytes do not appear to be of major importance in maintaining the structural integrity of the hair follicle. Of the many trichocyte or "hard" keratin genes that make up the hair shaft itself, only two have been linked to the dominant disorder monilethrix (Irvine and McLean, 1999). One sequence variation in the K6hf gene was reported previously in association with loose anagen syndrome (Chapalain *et al*, 2002), but this has not been independently confirmed.

In this issue, Winter *et al* describe a fairly common polymorphism in the K6hf gene. This sequence change leads to an amino acid substitution in the highly conserved helix initiation motif of the K6hf rod domain. This site is associated with severe disease phenotypes in the other keratins linked to genetic disease and so it is surprising at first to find this variation at a relatively high frequency in the population. The authors, however, went on to provide compelling evidence that this mutation/polymorphism is a predisposing factor for the development of the hair follicle abnormality pseudofolliculitis barbae (PFB). PFB is a common hair disorder typically characterized by ingrown hairs and associated inflammatory pustules and papules in areas that are subjected to regular shaving. Males are obviously more often affected in facial regions, particularly on the neck and submental areas where shaving is often performed against the orientation of the hair follicles. Females tend to be affected more often in areas where shaving takes place, i.e. axillae, pubic region and legs, although hirsute women who shave may also have a facial distribution of lesions. The incidence of PFB varies widely according to ethnicity, being much more common in Afro-

**Table I. Human keratin disorders to date**

| Keratin | Human genetic disorder(s) |
|---|---|
| K5/K14 | Epidermolysis bullosa simplex: (Dowling-Meara; Köbner; Weber-Cockayne; and recessive types) |
| K1/K10 | Bullous congenital ichthyosiform erythroderma (epidermolytic hyperkeratosis); K1 keratoderma; Striate keratoderma; Ichthyosis hystrix of Curth-Macklin |
| K9 | Epidermolytic palmoplantar keratoderma |
| K2e | Ichthyosis bullosa of Siemens |
| K6a/K16 | Pachyonychia congenita type 1 |
| K6b/K17 | Pachyonychia congenita type 2; Steatocystoma multiplex |
| K4/K13 | White sponge nevus of Cannon |
| K3/K12 | Meesman epithelial corneal dystrophy |
| K8/K18 | Cryptogenic cirrhosis |
| hHb6; hHb1 | Monilethrix |
| K6hf | Loose anagen syndrome (?); Pseudofolliculitis barbae |

American men ( $\sim$ 94% reported here) compared to Caucasian men ( $\sim$ 16%).

The A12T polymorphism in K6hf was found to co-segregate with PFB in a small family with an inheritance pattern consistent with dominant transmission, although this in itself was not statistically significant. Winter *et al* however, went on to look for the polymorphism in a large population of people who for reasons of their occupation, are forced to shave regularly. This showed that 76% of regularly shaving men who carry the A12T polymorphism have PFB making this a significant risk factor in the development of the disease. Carriers of the A12T polymorphism are greater than six times more likely to develop PFB compared with people homozygous for the wild-type K6hf sequence. Curly hair is another important factor in the development of ingrown hairs in PFB since curved hairs within shaved follicles are more likely to in-grow. Winter *et al* also show that curly hair increases the likelihood of PFB by a factor of 50 or more.

So how does the K6hf variation predispose to PFB? The authors postulate that close shaving sometimes produces hairs with sharp ends. In a person with curly hair, the curvature increases the likelihood that these hairs will in-grow. The fact that the companion cell layer is structurally weakened due to the K6hf mutation, which the authors clearly demonstrate to be disruptive to the keratin cytoskeleton, further increases the chances that a hair will in-grow. This hypothesis seems very plausible, however, some recent data from the same research group in collaboration with Pierre Coulombe's laboratory in Baltimore, revealed that K6hf is also expressed in the medulla of the hair shaft itself in murine pelage hairs and vibrissae (Wang *et al*, 2003). In humans, and of significance in relation to PFB, K6hf expression in the medulla is only seen in beard hairs and not in scalp hair, the latter being non-medullated hairs (Wang *et al*, 2003). Thus, it is conceivable that the abnormal K6hf expressed in the medulla of beard hairs might somehow make the hair more fragile and prone to forming ragged or sharp ends upon shaving and therefore further contribute to the pathogenesis of PFB. Another feature of keratin disorders is that the affected epithelial tissues tend to develop

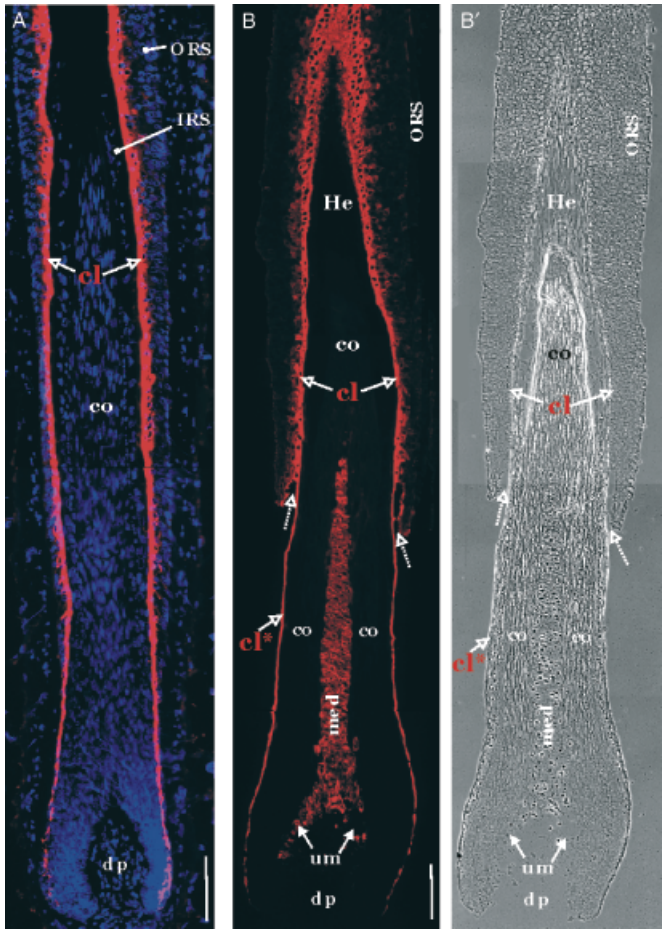


**Figure 1**
Immunofluorescence microscopy of K6hf in human hair follicles. Fresh frozen sections were reacted with a guinea pig antiserum specific for hK6hf followed by a Cy3-conjugated secondary antibody. Nuclei were revealed by incubation with DAPI. (*A*) Scalp hair follicle, which is typically non-medullated. K6hf is restricted to the companion layer. (*B*), (*B*') Fluorescence and phase contrast imaging of a beard hair follicle showing a prominent medulla compartment. In this instance, the upper matrix (um) and medulla (med) are strongly immunoreactive in addition to the companion layer (cl). Co = cortex; dp = dermal papilla; He = Henle layer; IRS = inner root sheath; ORS = outer root sheath. Illustration from Wang *et al* (2003), with permission.

hyperkeratosis (Irvine and McLean, 1999). It would be interesting to see if there is hyperkeratosis in the companion cell layer in people carrying the K6hf A12T variant, however, there are obvious difficulties in obtaining facial biopsy material and so this question remains unanswered.

The idea of keratin mutations/polymorphisms acting as genetic factors in complex traits emerged in recent years following the discovery of sequence variants in the simple epithelial keratins K8 and K18 in patients with cirrhosis and, more recently, inflammatory bowel disease (Coulombe and Omary, 2002; Owens and Lane, 2003). Thus, mutations in these important structural proteins, acting in concert with environmental lifestyle factors and other genetic modifiers, are important contributing factors in multifactorial diseases such as PFB. With the large number of keratins expressed in the hair follicle, there is bound to be a fair measure of functional redundancy in the system, however, there is also a very broad spectrum of hair phenotypes within the normal human population. It is therefore likely that sequence variants in the major structural proteins of the hair, namely epithelial keratins, trichocyte keratin and keratin-associated proteins, will emerge as genetic factors contributing to the normal hair texture, as well as hair diseases such as PFB, in the near future.

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## References

Bawden CS, McLaughlan C, Nesci A, Rogers G: A unique type I keratin intermediate filament gene family is abundantly expressed in the inner root sheaths of sheep and human hair follicles. J Invest Dermatol 116:157–166, 2001

Chapalain V, Winter H, Langbein L, *et al*: Is the loose anagen hair syndrome a keratin disorder? A clinical and molecular study. Arch Dermatol 138:501–506, 2002

Coulombe PA, Omary MB: 'Hard' and 'soft' principles defining the structure, function and regulation of keratin intermediate filaments. Curr Opin Cell Biol 14:110–122, 2002

Irvine AD, McLean WH: Human keratin diseases: Increasing spectrum of disease and subtlety of phenotype–genotype correlation. Brit J Dermatol 140:815–828, 1999

Irvine AD, McLean WH: The molecular genetics of the genodermatoses: Progress to date and future directions. Br J Dermatol 148:1–13, 2003

Langbein L, Rogers MA, Praetzel S, Winter H, Schweizer J: K6irs1, K6irs2, K6irs3, and K6irs4 represent the inner-root-sheath-specific type II epithelial keratins of the human hair follicle. J Invest Dermatol 120:512–522, 2003

Owens DW, Lane EB: The quest for the function of simple epithelial keratins. Bioessays 25:748–758, 2003

Wang Z, Wong P, Langbein L, Schweizer J, Coulombe PA: Type II epithelial keratin 6hf (K6hf) is expressed in the companion layer, matrix, and medulla in anagen-stage hair follicles. J Invest Dermatol 121:1276–1282, 2003

Winter H, Langbein L, Praetzel S, *et al*: A novel human type II cytokeratin, K6hf, specifically expressed in the companion layer of the hair follicle. J Invest Dermatol 111:955–962, 1998