Enamel Anomalies in a Pachyonychia Congenita Patient with a Mutation in KRT16


TO THE EDITOR

KRT6A, KRT6B, KRT6C, KRT16, and KRT17 genes encode a subset of epithelial keratins (K6a, K6b, K6c, K16, and K17) that are normally expressed in the skin of the palms and soles, in nail and hair, and in the oral epithelium. Mutations in these genes lead to pachyonychia congenita (PC), a cutaneous disorder featuring palmoplantar keratoderma and nail dystrophy, and potentially oral leukokeratosis, follicular keratosis, cysts, hyperhidrosis, and dental defects. K6 proteins that tend to accumulate in the enamel rod sheaths located at the periphery of the rods (Duverger et al., 2018).

In rodents, K16 is produced by secretory-stage ameloblasts and accumulates in the enamel matrix (Figure 1a). Outside the Tomes’ processes, which correspond to the highly specialized apical end of the ameloblasts where enamel matrix deposition is coordinated, K16 was detected as parallel transverse bands within the enamel rods as well as in the interrod regions (Figure 1a, top inset). The persistence of K16 in mature human enamel was verified by immunostaining on polished sections of human third molars. K16 was detected in the inner portion of the enamel, close to the dentin-enamel junction where it stained the core of the enamel rods (Figure 1b), contrary to K6 protein, a substitution that falls at the masking of the epitope in the KRT16 gene.

In this patient with PC introduced in this study, a female in her early twenties, we were able to draw any conclusion on the potential involvement of KRT16 and KRT17 in dental health due to a low number of polymorphisms that had high enough frequency in the cohorts studied. Here we report enamel defects in a patient with PC with a mutation in the KRT16 gene carrying a p.Asn171Lys substitution in K6a (Duverger et al., 2018).

Abbreviations: ALC, ameloblast-like cell; PC, pachyonychia congenita

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structure of her enamel. Based on micro-computed tomography data, the overall shape and appearance of the third molars were normal, except for small defects at the enamel surface (Figure 1e). The thickness and mineral density of both dentin and enamel were also within a normal range (Figure 1f). These results suggest that these teeth would probably appear as normal under standard dental consultation.

To further investigate potential defects in the enamel of this patient, we analyzed the ultrastructure of their extracted third molars using scanning electron microscopy. Sections performed in a plane transverse to the orientation of the rods revealed altered arrangement of the enamel rods and the presence of thin cracks going through multiple adjacent rods (Figure 2a). On mesial-distal sections, showing both the enamel layer and the underlying dentin, atypical cracks running transversely to the axis of the enamel rod were found in the inner enamel (Figure 2b). In a healthy tooth, cracks would normally propagate along the axis of the enamel rods.

To examine the effect of the K16 p.Asn125Ser mutation on the distribution of the protein in the enamel, we performed the immunohistochemical analysis of K16 on a polished third molar from the patient with PC. We found that mutant K16 tends to form aggregates of different sizes within the inner enamel (Figure 2c). Moreover, these aggregates were found at very high density in the transverse cracks shown in Figure 2b (Figure 2c, right panels). These results strongly support the idea that the cracks present in the inner enamel of this patient with PC are directly associated with the presence of mutant K16 proteins in the tissue.

Although it is unclear how K16 is secreted by ameloblasts in vivo and incorporated into the enamel matrix, we tested the behavior of the mutant K16 isoform in the context of a cellular system by overexpressing it in ameloblast-like cells (ALCs). Wild-type
KRT16 and KRT16 c.374A>G were cloned into a bidirectional vector allowing for tetracycline inducible co-expression with GFP (pBiGFP-KRT16WT producing K16WT and pBiGFP-KRT16A374G producing K16N125S) (Figure 2d). ALCs transfected with pBiGFP-KRT16 constructs produce GFP and K16 only when doxycycline is added to the culture medium (Figure 2e). Six hours after induction, while K16WT formed keratin filaments of various thicknesses and lengths in ALCs, K16N125S formed small aggregates that were distributed both in the cytoplasm and in the nucleus (Figure 2f). Even though this cellular assay does not reflect the process through which K16 is incorporated into the enamel in vivo, these results confirm that the Asn125Ser substitution in K16 results in the altered assembly of keratin filaments that tend to form aggregates, which is clearly distinct from the assembly of K16WT. When transgene expression was maintained for 24 hours, no cells exhibited K16N125S expression, whereas transgene expression could still be detected in ALCs that were transfected with K16WT. This suggests that K16N125S potentially has a toxic effect on ALCs when overexpressed. However, it is unlikely that the mutation results in such toxicity in ameloblasts in vivo as this would result in much more severe enamel defects.

This report further demonstrates the presence of keratins in tooth enamel, a notion that was long suggested based on the biochemical properties of the insoluble material that persists in mature enamel (Lesot et al., 1988; Robinson and Hudson, 2011; Robinson et al., 1975, 1989), and that we recently clarified with the identification of specific epithelial hair keratins (Duverger et al., 2014) and epithelial keratins (Duverger et al., 2018) in the tissue (Supplementary Figure S1 online). Our previous findings highlighted the importance of certain keratins (i.e., KRT6 and KRT75) in stabilizing the enamel rod sheaths to increase enamel resistance to caries (Duverger et al., 2014, 2018), a property of the enamel rod sheath that has long been supported (Little, 1962; Pincus, 1948). The present report emphasizes more the importance of other keratins (i.e., KRT16) to the biomechanical properties of enamel and more specifically the resistance to cracks, a property of the organic material present in mature enamel that has also been demonstrated (Baldassarri et al., 2008; Chai et al., 2009).
In epidermal tissues, K6 proteins (type II, basic or neutral) form heterodimers with K16 or K17 (type I, acidic) to assemble in larger polymeric structures. The fact that the distribution of K16 and K17 proteins is distinct from that of K6 in the enamel suggests that they follow an unconventional mode of assembly in this tissue that may involve interaction with other enamel matrix constituents. This assembly mode, as well as the mechanism through which these cytoskeletal proteins are incorporated into the enamel matrix, remains to be elucidated and is the focus of active investigation.

Altogether, our previous and present findings should increase the awareness that the development of closer collaborations between dermatologists and dentists would allow for proper monitoring and treatment of patients with keratin-associated cutaneous disorders.

**CONFLICT OF INTEREST**
The authors state no conflict of interest.

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**SUPPLEMENTARY MATERIAL**
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2018.07.005.

**REFERENCES**

**Epidermodysplasia Verruciformis: Genetic Heterogeneity and EVER1 and EVER2 Mutations Revealed by Genome-Wide Analysis**

**TO THE EDITOR**
Epidermodysplasia verruciformis (EV) (OMIM 226400) is a rare autosomal recessive genodermatosis characterized by susceptibility to cutaneous infections with β-human papillomaviruses (HPVs), with particular propensity to developing cutaneous malignancies (Lutzner et al., 1984). Characteristically, the early manifestations consist of thin tinea versicolor-like plaques and flat warts during childhood. Not infrequently, pigmented, papillomatous, and verrucous lesions may be found on the forehead or trunk. In their 30s and 40s, patients develop nonmelanoma skin cancers, mostly squamous cell carcinomas. Carcinogenesis is apparently caused by EV-HPV infection, particularly members of the β subfamily, and the majority of patients harbor EV-HPV types 5 and 8, but types 20 and 14 have also been detected, and a broader range of HPV types appears to be present in atypical EV (Imahorn et al., 2017; Lutzner et al., 1984; Orth, 2008). EV can be considered to be “typical,” as originally described by Lewandowsky and Lutz in 1922 (cited...