Hair keratin mutations in tooth enamel increase dental decay risk

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Tooth enamel is the hardest substance in the human body and has a unique combination of hardness and fracture toughness that protects teeth from dental caries, the most common chronic disease worldwide. In addition to a high mineral content, tooth enamel comprises organic material that is important for mechanical performance and influences the initiation and progression of caries; however, the protein composition of tooth enamel has not been fully characterized. Here, we determined that epithelial hair keratins, which are crucial for maintaining the integrity of the sheaths that support the hair shaft, are expressed in the enamel organ and are essential organic components of mature enamel. Using genetic and intraoral examination data from 386 children and 706 adults, we found that individuals harboring known hair disorder–associated polymorphisms in the gene encoding keratin 75 (KRT75), KRT75A161T and KRT75E337K, are prone to increased dental caries. Analysis of teeth from individuals carrying the KRT75A161T variant revealed an altered enamel structure and a marked reduction of enamel hardness, suggesting that a functional keratin network is required for the mechanical stability of tooth enamel. Taken together, our results identify a genetic locus that influences enamel structure and establish a connection between hair disorders and susceptibility to dental caries.

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Hair keratin mutations in tooth enamel increase dental decay risk

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Development of tooth enamel involves epithelial amelocytes, which secrete the organic matrix of tooth enamel. While keratins play a critical role in hair development, little is known about the keratin expression in ameloblasts. Previous studies have shown that keratin mutations are related to dental caries, yet the protein composition of tooth enamel has not been fully characterized. Here, we determined that epithelial ameloblast keratin, which are crucial for maintaining the integrity of the sheaths that support the hair shaft, are expressed in the enamel organ and are essential organic components of mature enamel. Using genetic and intraoral examination data from 386 children and 706 adults, we found that individuals harboring known hair disorder–associated polymorphisms in the gene encoding keratin 75 (KRT75), KRT75A161T and KRT75 E337K, are prone to increased dental caries. Analysis of teeth from individuals carrying the KRT75A161T variant revealed an altered enamel structure and a marked reduction of enamel hardness, suggesting that a functional keratin network is required for the mechanical stability of tooth enamel. Taken together, our results identify a genetic locus that influences enamel structure and establish a connection between hair disorders and susceptibility to dental caries.

Introduction

Dental caries are initiated at the surface of the tooth by microorganisms metabolizing food residues and releasing acids that dissolve enamel minerals (1). Their development is influenced by nutrition, hygiene, and genetic factors affecting the structure of tooth enamel (1–5).

During tooth development, amelogenin and other matrix components (enamelin, ameloblastin) are deposited by enamel-secreting ameloblasts in a highly structured manner to form enamel rods that are fully mineralized during maturation, with degradation of protein components and deposition of hydroxyapatite minerals (6). Mature enamel consists of approximately 96% minerals, 3% water, and 1% organic material by weight. Most of the organic material is located in the inner enamel where it forms very characteristic structures called enamel tufts due to their appearance at the dentin–enamel junction. Tuft material extends surrounding each individual rod (7). Enamel tufts and rod sheaths play an essential role in the mechanical performance of enamel (8, 9) and influence the initiation and progression of caries (10, 11). It has been suggested that they contain keratin-like material (12, 13), but a high degree of crosslinking has precluded full characterization of their protein composition (14).

While tooth and hair morphogenesis utilize common molecular pathways, the final structures produced are very different and involve specific structural components (15, 16). In the hair, hair keratins form filaments that are highly crosslinked and are major components forming the different layers of the hair shaft and supporting tissues, such as the inner root sheath, the companion layer, and outer root sheath. Here, we show that epithelial hair keratins are crucial structural components of human enamel and that mutations in these keratins increase the susceptibility to dental caries.

Results and Discussion

In a previous study, we showed that the transcription factor DLX3 directly controls the expression of hair keratins during hair follicle development (17). Performing transcriptome analysis on murine enamel organs lacking DLX3 (Krt14-Cre Dlx3−/−, herein referred to as Dlx3−/− mice at P10), we found that several epithelial hair keratins (Krt25, Krt27, Krt71, Krt75, and Krt76), expressed in the supporting tissues surrounding the hair shaft (inner root sheath and companion layer), were transcribed in the enamel organ and exhibited significantly downregulated expression in Dlx3−/− mice (Figure 1A and Supplemental Figure 1; supplemental material available online with this article; doi:10.1172/JCI78272DS1). In addition to previously detected KRT5 and KRT14, which are expressed in most epithelia, we detected the mRNA of other epithelial keratins in the enamel organ: Krt6a, Krt6b, Krt7, Krt10, Krt15, Krt16, Krt17, Krt19, and Krt80, none of which were significantly affected by Dlx3 deletion (Figure 1A and Supplemental Figure 1).

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hair in the facial area upon shaving, is associated with a polymorphism (rs2232387) in the KRT75 gene and more prevalent in African-Americans due to the curly nature of their hair (23). This G-to-A missense mutation (KRT75GA or KRT75 AA genotypes) leads to an alanine-to-threonine substitution at position 161 (A161T) in a highly conserved region of the 1A \( \alpha \)-helical segment of KRT75 (Figure 1E).

To determine whether the KRT75 A161T mutation had clinical impact on enamel function, we tested the genetic association between the KRT75 polymorphism (rs2232387) and tooth decay assessed as dental caries experience in the primary dentition of 386 children (mixed European descent, 6–12 years) and permanent dentition of 706 adults (mixed European descent, 25–50 years). The missense A allele of rs2232387, which was observed in 23% of the samples, was associated with a higher number of carious tooth surfaces in adults but not children (Figure 1F and Supplemental Table 1).

We investigated the expression pattern of KRT75 in mouse enamel organ and in mature human enamel. KRT75 was expressed by mouse ameloblasts at the secretory stage (Supplemental Figure 2A) and detected in the tufts and rod sheaths of mature human enamel (Figure 1, B and C). Interestingly, until enamel matrix proteins were identified, enamel was thought to be a keratinized structure, like hair and nail, with an extreme degree of mineralization (18, 19). Here, we show that a subset of hair keratins is expressed by ameloblasts, and we demonstrate that KRT75 is incorporated into the tufts and rod sheaths in mature enamel. Since keratins are not secreted proteins, their deposition in enamel may be related to the retention of cell fragments at the periphery of the rods (20).

Mutations in hair keratins have been reported in various human pathological conditions (21, 22). Among them, pseudofolliculitis barbae, a hair disorder characterized by the formation of ingrown hair in the facial area upon shaving, is associated with a polymorphism (rs2232387) in the KRT75 gene and more prevalent in African-Americans due to the curly nature of their hair (23). This G-to-A missense mutation (KRT75GA or KRT75 AA genotypes) leads to an alanine-to-threonine substitution at position 161 (A161T) in a highly conserved region of the 1A \( \alpha \)-helical segment of KRT75 (Figure 1E).

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el rods revealed that the distribution and characteristic keyhole
shape of the rods were altered in individuals carrying the variant
A allele (Figure 2A). Tufts and rod sheaths isolated from patients
with the $\text{KRT75 GA}$ or $\text{KRT75 AA}$ genotype appeared disorganized
(Figure 2B), and the distribution pattern of KRT75 was highly
disrupted (Figure 2C). This was further corroborated by trans-
mission electron microscopy analysis (Figure 2D). These results
suggest that epithelial hair keratins stabilize enamel tufts and rod
sheaths to support enamel rods during their formation, which is
similar to their function in supporting the hair shaft (23, 28, 29).

The functional role of KRT75 in teeth was further supported by
a mouse model ($\text{Krt75tm1Der}$) carrying a $\text{Krt75}$ mutation leading to
hair defects (30) as well as defects in the assembly of enamel rods
(Supplemental Figure 2, B–E).

To evaluate the mechanical consequences of these enamel
defects, we performed microhardness testing on molars from 6
individuals (3 of mixed European descent and 3 African-Amer-
icans) of each genotype ($\text{KRT75GG}$, $\text{KRT75GA}$, and $\text{KRT75 AA}$).
The missense variant in $\text{KRT75}$ led to a dose-dependent decrease
in the hardness of inner enamel, where organic material is more

Association with a second rarer missense polymorphism in
$\text{KRT75}$ (rs2232398) leading to a glutamate-to-lysine substitution at
position 337 (E337K) was shown previously for loose anagen hair
syndrome (24). In our cohort, this polymorphism was observed in
1% of the samples and was associated with increased caries in chil-
dren only (Supplemental Table 2). The 2 missense polymorphisms
were not in linkage disequilibrium, and in adults, there was mod-
est evidence of a statistical interaction between them (Supplemen-
tal Table 3), with one participant having both risk variants and 30
decayed tooth surfaces. Results were unchanged after adjusting for
tooth-brushing frequency and fluoride exposure. Altogether, these
results indicate that genetic variation in $\text{KRT75}$ increases the risk
dental caries and that the effects of specific polymorphisms may
differ across dentitions and by genotype at other loci. $\text{KRT75}$ can
therefore be added to the list of enamel formation genes (amel-
genin, ameloblastin, enamelin, tuftelin) in which polymorphisms
have been previously associated with caries experience (25-27).

To determine the functional effects of the missense polymor-
phism in $\text{KRT75}$ on enamel, we conducted structural analysis of
human enamel from individuals carrying the rs2232387 polymor-
phism. Scanning electron microscopy analysis of polished and
etched tooth samples sectioned in the plane transecting the enam-
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Figure 2. Effects of polymorphism in $\text{KRT75}$ on enamel and tufts structure and on enamel hardness. (A) Scanning electron microscopy analysis of
ground, polished, and etched human molars from patients with $\text{KRT75 AA}$ or without $\text{KRT75 AA}$ a missense A allele at rs2232387. Asterisk, rod; arrow, interrod. Scale bar: 20 μm. (B) Differential interference contrast imaging of tufts isolated from molars from individuals with $\text{KRT75 AA}$ or $\text{KRT75 AA}$ genotype. Scale bar: 500 μm. (C) Detection of KRT75 protein in tufts from individuals with $\text{KRT75 AA}$ or $\text{KRT75 AA}$ genotype. Scale bar: 20 μm. (D) Transmission electron microscopy of organic material isolated after demineralization of enamel from individuals with $\text{KRT75 AA}$ or $\text{KRT75 AA}$ genotype. Lower panels show high resolution images of enamel rod sheaths. Scale bars: 10 μm (upper panels); 500 nm (lower panels). (E) Vickers enamel hardness (adjusted for race) measured on molar sections from individuals with $\text{KRT75 AA}$, $\text{KRT75 AA}$, or $\text{KRT75 AA}$ genotype. n = 6 (3 of mixed European descent and 3 African-Amer-
icans) for each group.
Scanning electron microscopy analysis of the polished specimens used for microindentation revealed the presence of cracks and holes (20–100 μm in diameter) in the enamel from several individuals carrying the rs2232387 polymorphism (Figure 3A). The area surrounding the holes was hypomineralized, as indicated by the reduction in density (Figure 3A, arrow), and spherical particles observed in these holes suggested the presence of bacteria (Figure 3A, arrowhead), which was confirmed by Gram staining (Supplemental Figure 4). Micro-computed tomography (micro-CT) analysis showed significant differences in inner enamel hardness between participants of mixed European descent and African-Americans for all 3 genotypes considered (Supplemental Figure 3), and KRT75 genotype and race together accounted for 71% of variation in the inner enamel hardness (Supplemental Table 4). However, in contrast to pseudofolliculitis barbae (23), the clinical outcome of the rs2232387 polymorphism in terms of enamel defects and susceptibility to caries does not depend on race.
of molars revealed the presence of tubular projections of the pit and fissure area into the enamel. These structures were shallow in individuals with KRT75G6T; however, they penetrated through the enamel in individuals carrying the missense A allele (Figure 3, B and C). In 3 of the carriers analyzed, the number of these tubules was very high (10 to 20), and most of them reached the dentin (Figure 3C and Supplemental Videos 1–6). These tubular lesions, which possibly form along a group of prisms (31), may have serious clinical implications, such as delayed diagnosis of decay, due to lack of visible signs on the tooth surface and a fast progression of the disease into the dentin. Enamel rod boundaries have been acknowledged as the main pathway for acid attack and demineralization, and the proteins located at the rod sheath were shown to be more chemically stable in enamel from patients with healthy teeth than in sound enamel areas from patients who are prone to develop caries (10, 11). Moreover, the pit and fissure area of mature permanent human teeth has a very convoluted prism arrangement with high concentration of tufts (7) and is normally resistant to acid/carious attack (32), suggesting that tufts offer some protection against caries. Our results suggest that tufts and rod sheaths destabilized by the presence of KRT75G6T mutant protein have a reduced capacity to protect against caries.

This study provides the first characterization, to our knowledge, of specific epithelial hair keratins present in mature enamel and links hair disorder-associated polymorphisms in the KRT75 gene to increased susceptibility to dental defects and caries. We anticipate that other mutations in epithelial hair keratins will show a similar association.

Methods

Further information can be found in Supplemental Methods.

Statistics. Linear regression was used to test the association of genetic polymorphisms with dental caries experience and enamel hardness. A P value of less than 0.05 was considered significant. Data are expressed as mean ± SEM, and error bars denote SEM.

Study approval. Informed consent was provided by all participants or their guardians. All procedures, forms, and protocols were approved by NIAMS, the Institutional Review Boards of the University of Pittsburgh, West Virginia University, and the Walter Reed National Military Medical Center. All animal work was approved by the NIAMS Animal Care and Use Committee.

Accession numbers. All original completed microarray data and RNA-Seq data were deposited in the NCBI’s Gene Expression Omnibus (GSE57984). The genotype and phenotype data from the Center for Oral Health Research in Appalachia (COHRA) study cohort are available in the NCBI’s database of Genotypes and Phenotypes (dbGaP) (phs000095.v2.p1).

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