Published in final edited form as:
Cells Tissues Organs. 2009 ; 189(1-4): 224–229. doi:10.1159/000151378.

Human and Mouse Enamel Phenotypes Resulting from Mutation or Altered Expression of *AMEL, ENAM, MMP20* and *KLK4*

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Abstract

The amelogenesis imperfectas (AI) are caused by *AMEL, ENAM, MMP20* and *KLK4* gene mutations. Mice lacking expression of the *AmelX, Enam* and *Mmp20* genes have been generated. These mouse models provide tools for understanding enamel formation and AI pathogenesis. This study describes the AI phenotypes and relates them to their mouse model counterparts. Human AI phenotypes were determined in a clinical population of AI families and published cases. Human and murine teeth were evaluated using light and electron microscopy. 463 individuals from 54 families were evaluated and mutations in the *AMEL, ENAM, and KLK4* genes were identified. The majority of human mutations for genes coding enamel non-proteinase proteins (*AMEL* and *ENAM*) resulted in variable hypoplasia ranging from local pitting to a marked, generalized enamel thinning. Specific *AMEL* mutations were associated with abnormal mineralization and maturation defects. *Amel* and *Enam* null murine models displayed marked enamel hypoplasia and a complete loss of prism structure. Human mutations in genes coding for the enamel proteinases (*MMP20* and *KLK4*) cause variable degrees of hypomineralization. The murine *Mmp20* null mouse exhibits both hypoplastic and hypomineralized defects. The currently available *Amel* and *Enam* mouse models for AI exhibit enamel phenotypes (hypoplastic) that are generally similar to those seen in humans. *Mmp20* null mice have a greater degree of hypoplasia compared with humans having *MMP20* mutations. Mice lacking expression of the currently known genes associated with the human AI conditions provide powerful models for understanding the pathogenesis of these conditions.

Keywords

enamel; amelogenesis imperfecta; mouse; human; gene; mutation

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Introduction

The amelogenesis imperfectas (AI) are a clinically and genetically diverse group of conditions caused by mutations in genes critical for normal enamel formation. Mutations in the *AMELX*, *ENAM*, *MMP20* and *KLK4* genes are associated with specific AI types having X-linked, autosomal dominant and autosomal recessive modes of inheritance (Wright 2006). The pathogenesis of these conditions and the developmental mechanisms leading to the specific phenotypes remains poorly understood. This is largely due to the lack of adequate cell models for studying the complexities of enamel formation. Given the uniqueness of enamel development and the cell specific expression of many of the genes involved in tooth development in general and the enamel in particular, identification and functional characterization of enamel forming genes in humans is significantly limited. The generation of animal models provides an important resource to study normal and abnormal enamel development. Generation of mice lacking expression of genes associated with enamel formation provides a potentially powerful tool for understanding biomineralization of enamel and the pathogenesis of the different AI types (Gibson et al. 2001; Caterina et al. 2002).

Amelogenin is the most abundant extracellular matrix (EC M) protein in developing enamel. Amelogenins are encoded for by two single copy genes chromosome Xp22.3-p22.1 and on chromosome Yp11 (Salido et al. 1992; Fincham and Simmer 1997). Mutations in the X chromosome amelogenin gene (*AMELX*) cause a variety of changes in the amelogenin protein and are associated with AI phenotypes ranging from hypoplastic to hypomineralized enamel (Wright et al. 2003). Enamelin is a relatively low abundance matrix protein in developing enamel and is coded for by the *ENAM* gene which is located within a cluster of genes critical to biomineralization on chromosome 4q21 (Hu and Yamakoshi 2003). Mutations in *ENAM* cause AI types characterized by localized pitted enamel or generalized thin enamel (Rajpar et al. 2001; Mardh et al. 2002). MMP20 and KLK4 are proteinases critical for processing the enamel matrix thereby allowing the enamel crystallites to grow into space previously occupied by the extracellular matrix (Simmer and Hu 2002). The genes coding for these proteins are located on chromosomes 11q23 and 19q13 respectively and are associated with autosomal recessive forms of AI. Abnormal proteinase activities result in hypomaturation AI that is characterized by enamel that is deficient in mineral content but is of normal enamel thickness (Hart et al. 2004; Kim et al. 2005). Despite extensive studies as to how these proteins orchestrate the biomineralization of enamel, our knowledge of the complex processes that result in the structure and composition of enamel remains lacking. The purpose of this study was to compare and contrast the phenotypes of human enamels from individuals affected with AI to those mouse enamels that have been genetically modified by deletion of specific enamel matrix protein genes.

Methods

Human genotypes and phenotypes for AI were determined from a large clinical cohort that has been recruited to evaluate the etiology and pathogenesis of these conditions. This study was approved by the Institutional Review Board and all study participants provided informed consent prior to participation. DNA was collected from blood or saliva and candidate genes were sequenced using previously published techniques and primer sets for the *AMELX*, *ENAM*, *MMP20* and *KLK4* genes (Hart et al. 2002; Hart et al. 2003; Hart et al. 2004; Kim et al. 2005). All individuals were clinically evaluated by two examiners and the dentition photographed and dental radiographs taken whenever possible. Exfoliated primary or permanent teeth slated for therapeutic extraction were collected for histological analysis. The teeth were evaluated with light microscopy by cutting thin sections using a diamond blade. Samples were also cut, polished and etched or fractured for evaluation using scanning electron microscopy. Mice lacking expression of *Amelx*, *Enam*, and *Mmp20* have been generated in the

laboratories of several authors and have been described previously (Gibson et al. 2001; Caterina et al. 2002). The teeth from these animals were examined using light and scanning electron microscopy. Sample preparation was similar to that for the human samples. The gross and histological enamel phenotypes of the mouse and human were compared

Results

Families segregating for the AI trait were recruited and a total of 463 individuals from 54 families were enrolled. Coding region including intron-exon junctions of candidate genes (*AMELX, ENAM, AMTN, AMBN, MMP20 and KLK4*) were priority sequenced based initially on reported phenotype-genotype relationships and if no mutation was identified then remaining candidate genes were sequenced. Mutations were identified in the *AMELX, ENAM, and KLK4* genes and no mutations were identified in *MMP20*, *AMTN* (amelotin) or *AMBN* (ameloblastin) genes.

The majority of human mutations for genes coding non-proteinase enamel proteins (*AMELX* and *ENAM*) resulted in variable degrees of hypomineralization and hypoplasia that ranged from pitting and grooves to a marked, generalized thinning of the enamel. Three different *AMELX* mutations were identified. Two mutations (*AMLEX* g.3458delC, *AMELX*g.4046delC) were associated with enamel that was of normal or nearly normal thickness with a prismatic structure and decreased mineral content apparently due to maturation defects. The third *AMELX* mutation resulted in loss of the C-terminus and a hypoplastic phenotype (*AMELX*g.4046delC) (Figure 1). In addition to being markedly decreased in thickness the enamel lacked a prismatic architecture. Females having these mutations showed a mosaic phenotype with areas of more normal enamel adjacent to regions of more affected (thin/hypomineralized) enamel. Two different *ENAM* mutations were identified (*ENAM* g.8344delG; g.13185_13186insAG) and both were associated with generalized thin enamel that had a complete lack of prism structure (Figure 1). The thin layer of enamel had a rough surface and lacked any evidence of a prismatic structure. Backscatter analysis showed a laminated type of enamel pattern. Human mutations in genes coding for the enamel proteinases (*MMP20* and *KLK4*) cause variable degrees of hypomineralization but appear to have a normal enamel thickness based on radiographic assessment (Hart et al. 2004;Kim et al. 2005;Ozdemir et al. 2005). No MMP20 mutations were identified in this study population and there have been no histological evaluations reported of MMP20 enamel.

Murine KO models involving *Amel* and *Enam* genes are both associated with marked enamel hypoplasia and a complete loss of prism structure (Figure 2). The mandibular incisors in both these models show a loss of the typical yellow brown coloration seen in the WT mice. The enamel surface in both the *AmelX* and *Enam* null mice is rough compared with the WT enamel. The *AmelX* null mouse enamel is reduced from approximately 100 μm to 10 μm and shows no prismatic structure. The *Enam* null mouse enamel is only a few microns thick and shows no organization into prism structure (Figure 2). The murine *Mmp20* null mouse exhibits both hypoplastic and hypomineralized defects and shows areas of enamel loss (Caterina et al. 2002).

Discussion

The enamel phenotypes associated with AI in humans are diverse and range from a decrease in the amount of enamel to enamel that is of normal thickness but lacks the high level of mineralization seen in normal enamel. Both of these phenotypes have been reproduced in mouse models that have been generated to perturb the normal expression of essential extracellular matrix proteins involved in amelogenesis (Gibson et al. 2001; Seedorf et al. 2007). The AI associated enamel phenotypes in humans and mice appear to differ depending

on whether the mutation/knock out involves genes encoding for an ECM protein (e.g. amelogenin or enamelin) versus the ECM proteinases (matrix metalloproteinase 20 and Kallikrein 4). The currently available *Amelx* and *Enam* mouse models for AI exhibit enamel phenotypes (hypoplastic) that are generally similar to those seen in humans. There is a loss of a discernable prismatic architecture in both humans having mutations that cause a loss of amelogenin (signal peptide mutations) or loss of the C-terminus (Wright et al. 2003). Similarly the mice lacking amelogenin show no evidence of a prism structure (Gibson et al. 2001). Humans having *ENAM* mutations in the present study showed generalized thin enamel with a roughened surface similar to the *Enam* null mouse. The developmental mechanism in humans with ENAM mutations that cause localized pitted hypoplastic defects likely differ from that causing generalized hypoplasia in the *Enam* null mice (Mardh et al. 2002; Hart et al. 2003). Humans having *ENAM* mutations that cause haploinsufficiency are associated with localized hypoplasia whereas those having generalized hypoplasia are thought to represent a dominant negative effect. The Enam null mouse more closely resembles the latter defect but the lack of a laminated enamel appearance could be due to the differences in developmental mechanisms between the complete loss of protein (*Enam* null mouse) and the dominant negative mechanism in humans. Mice lacking expression of *Mmp20* have a greater degree of hypoplasia coupled with hypomineralization while humans with *MMP20* mutations tend to exhibit primarily hypomineralization defects. However, it is possible that humans with *MMP20* mutations could have varying levels of enamel hypoplasia as there have been no published histological studies of teeth from individuals with a known MMP20 mutation to our knowledge. While reasonably detailed analysis of human enamel affected by a *KLK4* mutation has been reported. there is no mouse model to study the specific mechanism of how this autosomal recessive trait results in the enamel phenotype observed (Hart et al. 2004).

The number of known human mutations in genes coding for the enamel extracellular matrix and proteinases continues to grow. There are well over 20 mutations in four genes and the present and other studies suggests there will be at least several additional AI associated genes identified in the near future. Of the 463 study participants studied from 54 families mutations were detected in only 9 kindreds. Linkage in two large families with autosomal dominant hypocalcified AI was identified to the 8q24.3 locus as has beenreported by others (Mendoza et al. 2007). The present study and many recent investigations excluding known candidate genes in the AI families tested suggests that the current candidate genes account for less than 70% of the AI cases. Continued clinical investigations are clearly indicated to identify new genetic loci and genes associated with AI and to characterize the variability and diversity of resulting phenotypes so we may better understand the role of these enamel related genes in amelogenesis.

Comparison of mouse and human pathogenesis caused by different perturbations in the genes responsible for generating the ECM of enamel shows marked similarities and some subtle differences. This may arise for several reasons. It is well known that animal models of human diseases do not always recapitulate the human disease phenotype. While many human mutations alter function of the normal protein product, most mouse models for AI candidate genes are designed to knock out the gene. Such null models can not fully recapitulate the functional range of AI associated human gene mutations. Additionally, the effect of possible epigenetic interactions is limited in current murine models, compared to the more diverse genetic background that exists in the human population studied to date. Development of mice that have gene changes similar to those in humans (e.g. point mutations, missense mutations, etc) may provide even better models for understanding the specific mechanisms involved in particular AI subtypes (Gibson et al. 2007). However, the current mouse models provide a powerful tool for helping understand the role of the genes and their ECM products in amelogenesis and biomineralization. This is especially true given the lack of cellular models for investigating amelogenesis and mineralization of this highly mineralized tissue.

Acknowledgments

Supported in part by NIDCR grants DE12879 (TW), DE011089 (CW), DE01627 (JB) DE11301 (JH).

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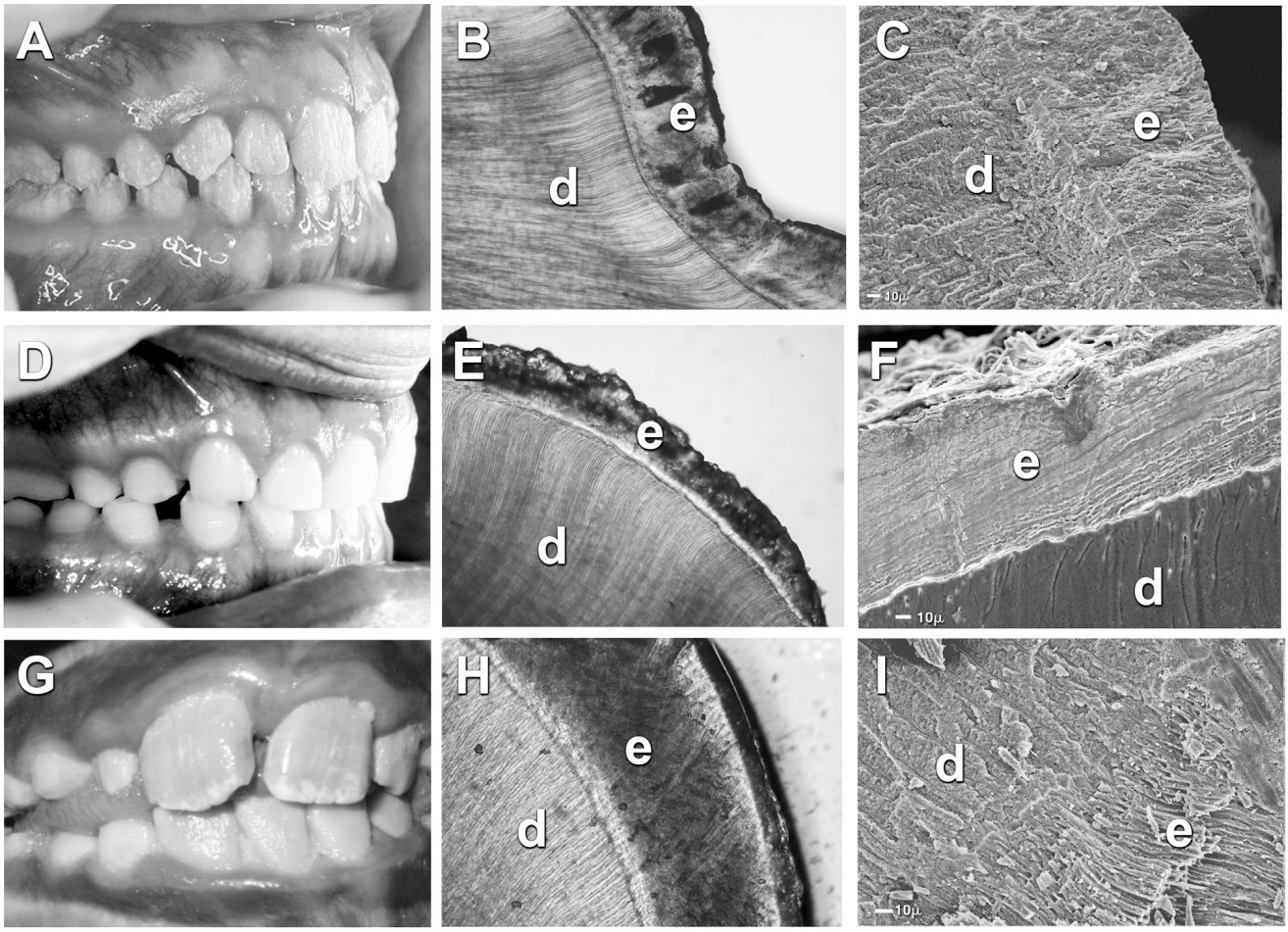

Figure 1.

Highly diverse clinical appearances of the human dentition result from *AMELX* (panel A shows female dentition), *ENAM* (panel D), and *KLK4* mutations (panel G). The dentin (d) appears normal while the enamel (e) affected by these different mutations shows varying degrees of opacity and hypoplasia as seen with light microscopy (panel B *AMELX*, panel E *ENAM*, panel H *KLK4*). These different mutations also have markedly different affects that frequently disrupt the normal prismatic structure in the *AMELX* (panel C) and *ENAM* (panel F) affected enamel but not in the *KLK4* AI enamel (panel I) as seen with scanning electron microscopy.

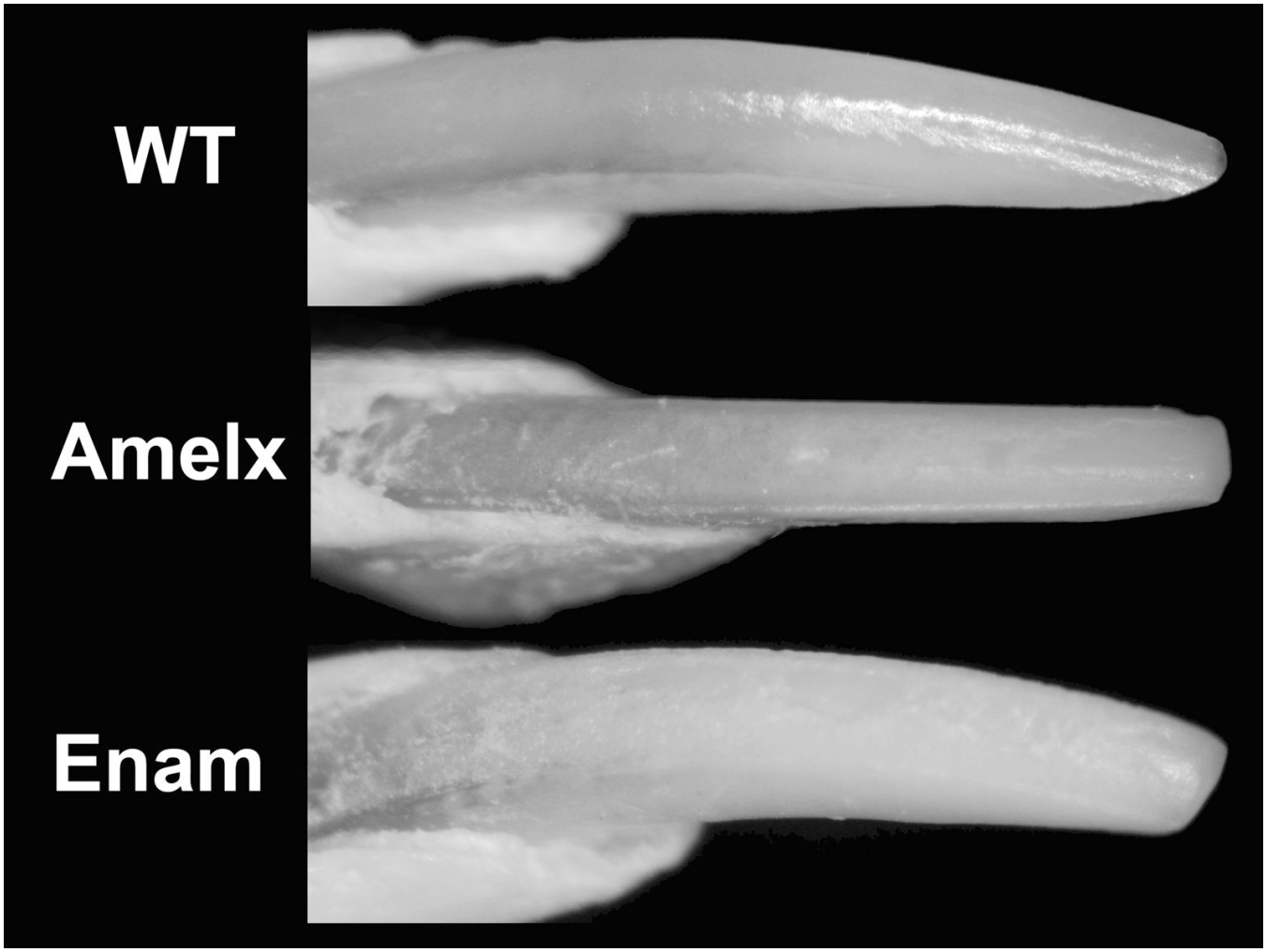

Figure 2.
Wild-type mice (WT) typically have smooth enamel with a yellowish-brown coloration. Mice null for *Amelx* and *Enam* show a roughened surface, abnormal wear on the incisal edge and a white opaque appearance.

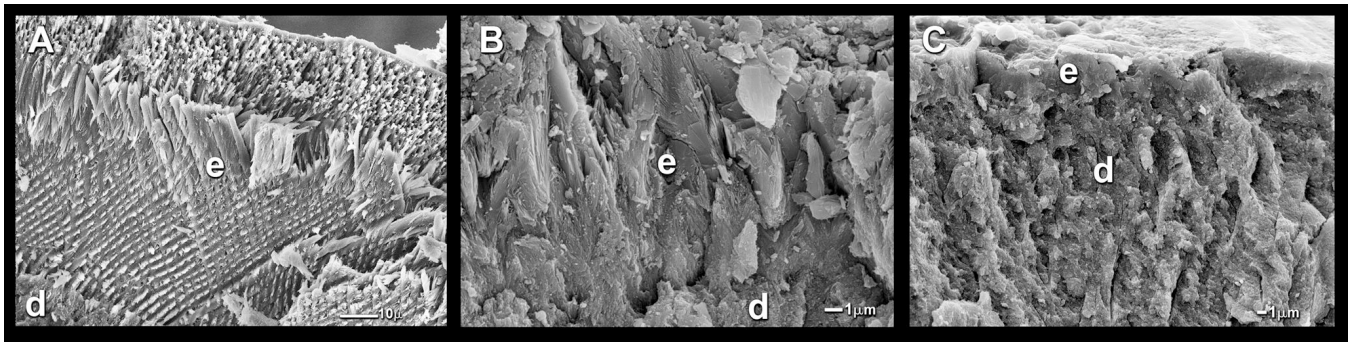

Figure 3.
Scanning electron microscopy reveals the typical prismatic enamel (e) architecture in the WT mouse (A) and the complete loss of prisms in both the Amelx-null (B) and Enam-null (C) mice. Similar to humans the dentin (d) appears structurally normal.