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JC-C Hu
JP Simmer

Developmental biology and genetics of dental malformations

Authors' affiliations:
Jan C-C. Hu, Department of Orthodontics and Pediatric Dentistry, University of Michigan School of Dentistry, Ann Arbor, MI, USA
James P. Simmer, Department of Biologic and Materials Sciences, University of Michigan Dental Research Lab, Ann Arbor, MI, USA

Correspondence to:
James P. Simmer
Department of Biologic and Materials Sciences
University of Michigan Dental Research Lab
1210 Eisenhower Place
Ann Arbor
MI 48108, USA
E-mail: jsimmer@umich.edu

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Structured Abstract

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The synthesis of tooth development biology with human studies focusing on inherited conditions that specifically interfere with tooth development is improving our understanding of normal and pathological tooth formation. The type of inherited dental malformations observed in a given kindred relate to when, during odontogenesis, the defective gene is critically expressed. Information about the protein encoded by the defective gene and the resulting dental phenotype helps us understand the major processes underway at different stages during tooth development. Genes affecting early tooth development (*PAX9, MSX1*, and *AXIN2*) are associated with familial tooth agenesis or oligodontia. Genes expressed by odontoblasts (*COL1A1*, *COL1A2*, and *DSPP*), and ameloblasts (*AMELX*, *ENAM*, *MMP20*, and *KLK4*) during the crown formation stage, are associated with dentinogenesis imperfecta, dentin dysplasia, and amelogenesis imperfecta. Late genes expressed during root formation (*ALPL* and *DLX3*) are associated with cementum agenesis (hypophosphatasia) and taurodontism. Understanding the relationships between normal tooth development and the dental pathologies associated with inherited diseases improves our ability to diagnose and treat patients suffering the manifestations of inherited dental disorders.

Key words: amelogenesis imperfecta; dentinogenesis imperfecta; hypophosphatasia; taurodontism; tooth agenesis

Introduction

Teeth are specialized structural components of the craniofacial skeleton and are comprised of three distinct mineralized tissues: enamel, dentin, and cementum. Developmental defects occur in each of these mineralized tissues, sometimes alone (isolated), and sometimes in combination (syndromic) with defects in other organs or tissues. Here we focus on developmental defects with phenotypes that are predominantly restricted to the dentition. For a genetic defect to cause dental anomalies restricted to teeth, it must be that the defective gene is critical for proper dental development, but is not critical or is less critical for the development of all other tissues and organs. Genes involved in the etiology of isolated amelogenesis imperfecta (AI), for instance, may be expressed in multiple tissues, but restriction of the disease phenotype to dental structures suggests that the proteins they express are specialized for tooth formation. When a

gene is normally expressed in multiple tissues, but defects in the gene are manifested as isolated dental malformations, the non-dental expression is either without function or functional redundancy allows other molecules to manage the deficit. It is also possible that the non-dental defects are subtle and go unrecognized, or are manifested only infrequently under extraordinary circumstances. In this report we concentrate on genetic diseases that cause isolated dental anomalies, including familial tooth agenesis, amelogenesis imperfecta (AI), dentinogenesis imperfecta, dentin dysplasia, hypophosphatasia, and taurodontism.

Early tooth development and familial tooth agenesis

Tooth development and morphogenesis (Fig. 1) occurs through a series of epithelial–mesenchymal interactions. The first signs of tooth development are focal condensations of migratory neural crest cells immediately beneath the oral epithelium of the future alveolar ridge (1, 2). The initiation of tooth formation involves the synthesis and secretion of diffusible growth factors by the oral epithelium (3–5), which induce the expression of transcription factors required for differentiation of the underlying ectomesenchyme. Msx1 and Pax9 are transcription factors expressed by the mesenchyme early in tooth formation. Msx1 and Pax9 knockout mice arrest tooth development at the bud stage (6, 7), and *MSX1* (4p16.3–p16.1) (8) and *PAX9* (14q12–q13) (9) gene mutations cause autosomal dominant familial tooth agenesis in humans. Only some of the teeth are missing in people with *MSX1* and *PAX9* mutations. The patterns of missing teeth in *MSX1* and *PAX9* kindreds are similar, but distinguishable (10). Axin2 is a Wnt regulator protein expressed by the ectomesenchyme. *AXIN2* (17q23–q24) mutations cause a severe form of autosomal dominant familial tooth agenesis associated with a high risk for colorectal cancer (11). Because of the link with cancer, genetic tests to rule out *AXIN2* involvement are advised for patients with familial tooth agenesis. Some genes involved in early tooth development cause familial tooth

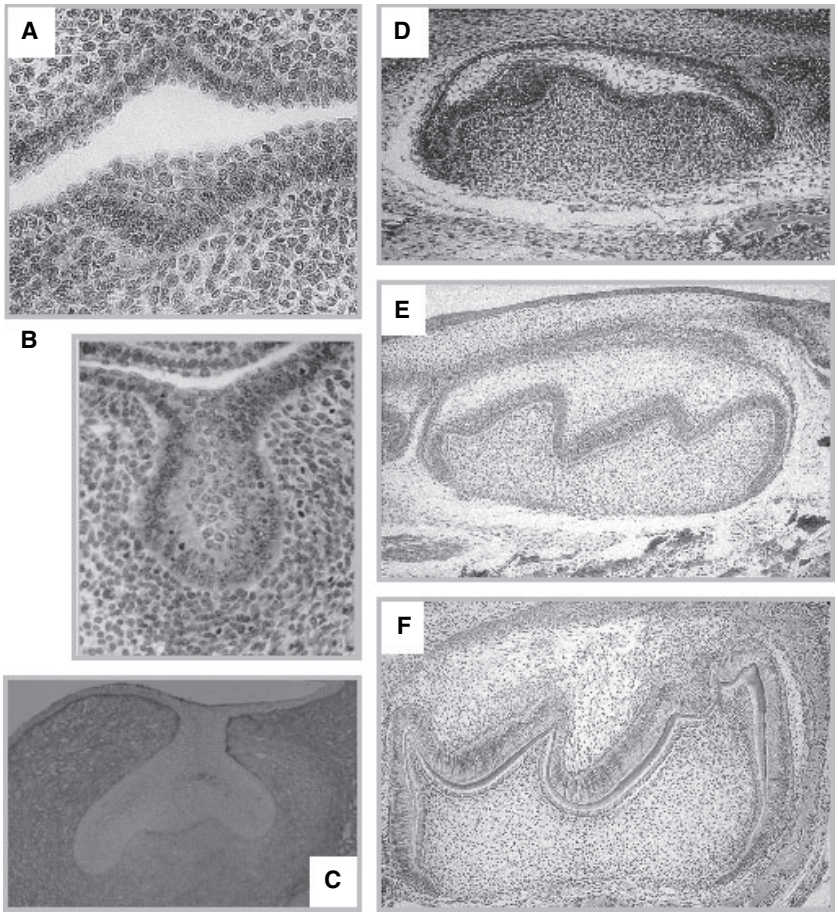

Fig. 1. Histological stages of tooth formation. (A) Initiation Stage, (B) Bud Stage, (C) Cap Stage, (D) Bell Stage (early), (E) Bell Stage (late) and (F) Crown Formation Stage. In early tooth formation epithelial–mesenchymal interactions direct tooth formation and loss of function mutations in the genes encoding relevant signaling molecules and transcription factors can lead to an arrest of tooth development. Defects in genes expressed during crown formation (F) can lead to enamel and dentin defects categorized as amelogenesis imperfecta, dentinogenesis imperfecta and dentin dysplasia.

agenesis as part of a larger syndrome, such as in ectodermal dysplasia (12, 13) or Rieger syndrome (14).

Early epithelial–mesenchymal interactions drive tooth morphogenesis through the initiation (Fig. 1A) and bud stages (Fig 1B) to form the dental papilla in the cap stage (Fig 1C). During the cap stage the signaling center that drives differentiation is the primary enamel knot (15). The enamel knot includes inner enamel epithelial cells that will be the first to differentiate into secretory ameloblasts at the future cusp tip of the developing tooth and its signals are believed to induce odontoblast differentiation, which is most advanced under the cusp tips. In bell stage molar teeth, secondary enamel knots are in the enamel epithelium at each cusp tip (Fig 1D) (16, 17). The essential shape of the crown is established through the series of epithelial-mesenchymal interactions prior to biomineralization. During the crown formation stage, terminally differentiated odontoblasts and ameloblast deposit dentin and enamel matrix proteins, respectively. These macromolecules assemble and organize the matrix, and induce and regulate mineralization of the matrix. In this way biomineralization is dependent upon genes that encode extracellular matrix components. Mutations in genes encoding specialized enamel and dentin extracellular matrix proteins are shown in Table 1.

Dentin formation and inherited dentin defects

The most abundant molecules in dentin are type I collagen and dentin sialophosphoprotein (DSPP) derived proteins. Shortly after DSPP is synthesized by odontoblasts, it is cleaved into three structural/functional domains: dentin sialoprotein (DSP), dentin glycoprotein (DGP), and dentin phosphoprotein (DPP). Collagen constitutes almost 90% of the dentin organic matrix (18), while dentin sialoprotein (DSP), dentin glycoprotein (DGP), and dentin phosphoprotein (DPP) constitute most of the non-collagenous constituents (19–22). Other non-collagenous proteins in dentin include dentin matrix protein 1 (DMP1) osteocalcin, osteonectin (SPARC), osteopontin (OPN) matrix gla protein (MGP), matrix extracellular phosphoglycoprotein (MEPE), decorin and biglycan, but the concentrations of these other proteins are very low in dentin compared with bone.

Table 1. **Mutations affecting enamel and dentin during crown formation. The mutation abbreviations in the first column refer to the predicted alteration of the protein, so p.T51I would mean threonine at position 51 is changed to isoleucine**

	Phenotypes	References
AMELX		
p.0	Hypomineralization	(54)
p.P52fsX53	Hypoplastic-hypomineralization	(55, 56)
p.I5-A8delinsT	Hypoplastic	(57)
p.T51I	Hypoplastic	(58)
p.E191X	Hypoplastic	(58)
p.P158fsX187	Hypomineralization	(58)
p.P70T	Hypomaturation	(59–61)
p.L181fsX187	Hypoplastic-hypomineralization	(62, 63)
p.H129fsX187	Pitted hypoplastic	(64)
p.W4X	Rough hypoplastic	(65)
p.H77L	Hypomaturation	(63)
p.Y141fsX187	Severe hypoplastic	(66)
p.W4S	Hypoplastic	(38)
p.M1T	Hypoplastic	(38)
ENAM		
p.A158-Q178del	Hypoplastic, AD	(67, 68)
p.K53X	Pitted-hypoplastic, AD	(33, 69)
p.N197fsX277	Hypoplastic, AD	(41, 70, 71)
p.S247X	Hypoplastic, AD	(43)
p.M71-Q157del	Hypoplastic, AD	(41)
p.V340_M341insSQYQYCV	Pitted-hypoplastic, AR	(42)
p.P422fsX448	Hypoplastic, AD	(42)
MMP20* and *KLK4		
MMP20 – g.IVS6-2A > T	Hypomaturation, AR	(48)
MMP20 – p.H226Q	Hypomaturation, AR	(49)
KLK4 – p.W153X	Hypomaturation, AR	(50)
DSPP		
p.Q45X	DGI II	(25)
p.P17T	DGI II	(26)
g.1275G > A (intron 3)	DGI II	(26)
p.V18F	DGI II, DGI III	(26, 30)
p.Y6D	DD II	(27)
p.A15V	DGI II	(28)
g.1188C > G (intron 2)	DGI II	(29)
p.R68W	DGI II	(28)
p.del1160–1171 and p.ins1198–1199	DGI III	(31)

Historically, inherited dentin defects have been classified as either dentin dysplasia (DD) types I and II, or dentinogenesis imperfecta (DGI) types I, II, or III (23). DGI type I is now universally designated as osteogenesis imperfecta with dentinogenesis imperfecta (OI/DGI), and is caused by type I collagen mutations (24). Among the genes expressing the non-collagenous proteins in dentin, only the *DSPP* gene has been implicated in the etiology of DD and DGI. To date, nine different *DSPP* mutations have been reported in kindreds with inherited dentin defects (25–31). Mutations in the *DSPP* gene have been shown to cause DD type II, DGI type II and DGI type III. The *Dspp*$^{-/-}$ mouse tooth defects resemble human DGI-III, which is a rare human phenotype (32).

Enamel formation and amelogenesis imperfecta

Inherited enamel defects that occur in the absence of a generalized syndrome are collectively designated as *amelogenesis imperfecta*. There are different clinical forms of AI and many genes are involved in its etiology. There are four proven candidate genes for AI: amelogenin (*AMELX*), enamelin (*ENAM*), enamelysin (*MMP20*), and kallikrein 4 (*KLK4*). These genes encode proteins secreted into the enamel matrix of developing teeth; however, mutational analyses of these candidate genes are only successful in finding a disease-causing mutation in about 25% of the AI kindreds studied (33). Thus more genes than have currently been implicated are likely to participate in the etiology of AI. A fifth candidate gene for AI, *DLX3*, causes AI as part of tricho-dento-osseous (TDO) syndrome (34). When the hair and bone abnormalities in TDO are subtle or not recognized, the condition is designated AI hypoplastic-hypomaturation with taurodontism (AIHHT) (35).

X-linked AI

X-linked AI accounts for about 5% of all AI cases (36), and is caused by defects in the amelogenin gene on the X-chromosome (Xp22.3–p22.1). There is a second amelogenin gene on the Y-chromosome (*AMELY*), but this gene is expressed at low levels and does not contribute to the etiology of AI. Amelogenin comprises 80 to 90% of the protein in developing enamel (37). To date, 14 different disease-causing mutations have been identified in *AMELX* (38), with different phenotypic patterns associated with mutations affecting three different regions of the amelogenin protein (39).

Autosomal dominant AI

The enamelin gene (*ENAM*, 4q13) encodes the largest enamel protein (*c.* 190 kDa), but this protein is the least abundant structural protein in the matrix (*c.* 3–5%). The *ENAM* gene has ten exons, eight of which are coding (40). To date, seven different disease-causing mutations have been identified in *ENAM* (41, 42). Single *ENAM* allele defects typically produce thin enamel, sometimes with horizontal grooves. In its most mild form, only small, well-circumscribed enamel pits are evident (43). When both *ENAM* alleles are affected, there is almost no enamel layer (42).

Autosomal recessive AI

There are two secreted proteolytic enzymes in developing enamel: enamelysin (*MMP20*, 11q22.3) (44, 45) and kallikrein 4 (*KLK4*, 19q13.41) (46, 47). Both were originally discovered in developing teeth (45, 47). Although these enzymes are expressed at different times during amelogenesis, defects in both genes cause autosomal recessive pigmented hypomaturation AI (48–50).

Genetic defects manifested late in tooth development

As noted earlier, *DLX3* defects cause tricho-dento-osseous syndrome. Besides the enamel defects, the molars associated with this condition have a root-shape deformation of multirooted teeth known as taurodontism. Taurodontism is a variation in tooth form in multirooted teeth in which the bifurcation or trifurcation of the roots is displaced toward the apex of the root, resulting in an increased size of the pulp chamber. The crowns and pulp chambers are unusually long and the roots short, so the molars exhibit a bull-like shape, with the horns being the roots.

Taurodontism can affect the primary and permanent dentitions. The epithelial–mesenchymal interactions governing root morphogenesis are poorly understood, so little is know about how *DLX3* mutations cause taurodontism.

Hypophosphatasia is a bone disorder caused by mutations in the liver/bone/kidney alkaline phosphatase (*ALPL*, 1p36.1–p34) (51). This enzyme hydrolyzes pyrophosphate (PP_i) preventing it from inhibiting hydroxyapatite crystal growth. Hypophosphatasia is usually recessive and shows a variable clinical expression. The greater the severity of the disease, the earlier the diagnosis is made. In extreme cases there is a complete absence of skeletal mineralization and the affected infant succumbs at birth. In contrast, childhood onset hypophosphatasia is often first recognized by pediatric dentists, who are consulted to explain the premature exfoliation of fully rooted primary teeth (52, 53). Histological examination of the avulsed teeth shows that they lack both cellular and acellular cementum.

Genetics of tooth development

Our understanding of the genetic basis of tooth development and dental defects has been advancing on many levels. Early events in tooth development depend upon epithelial–mesenchymal interactions that involve the secretion of diffusible signaling molecules that induce the expression of transcription factors in the responding tissue. Epithelial–mesenchymal interactions are a common means of organ differentiation and similar gene networks regulate the development of teeth and other organs. Functional failures involving key participants in these signaling systems arrest tooth development, and hypodontia and familial tooth agenesis are a feature of many syndromes. Because of their expression during the development of other tissues, it is perhaps surprising that the loss of potent transcription factors such as Pax9 and Msx1 would be manifested as non-syndromic familial tooth agenesis, with only some teeth being affected and the pattern of tooth agenesis varying among affected individuals in the kindred. Regardless of the broadness of the clinical manifestations, the key concept is that early tooth formation depends upon a network of signaling molecules and transcription factors that drive cell proliferation and differentiation. A series of epithelial–mesenchymal interactions ultimately leads to the terminal differentiation of odontoblasts and ameloblasts and mineralization of the tooth.

After the basic shape of the tooth crown is established, odontoblasts initiate their secretion of a collagen-based matrix beneath the basal lamina of pre-ameloblasts, which start, slowly at first, to secrete enamel matrix proteins. Much has been published about the roles of these extracellular matrix molecules, particularly amelogenin, in the final differentiation of odontoblasts and ameloblasts, but the genetic studies prove they serve no critical role in cell differentiation. Odontoblasts terminally differentiate normally and produce normal dentin in patients with AI caused by defined mutations in the amelogenin (*AMELX*), enamelin (*ENAM*), enamelysin (*MMP20*) and kallikrein 4 (*KLK4*) genes. Similarly, ameloblasts terminally differentiate and produce normal enamel in patients with osteogenesis imperfecta, dentinogenesis imperfecta, and dentin dysplasia caused by defined mutations in *COL1A1* (17q21.31–q22), *COL1A2* (7q22.1), and *DSPP*. The genetic evidence suggests that changes in odontoblast and ameloblast activities secondary to their exposure to enamel or dentin extracellular matrix molecules must be involved in late events, such as in synchronizing and fine-tuning secretions to meet the requirements of biomineralization.

Root formation starts after the crown has been defined. The inner and outer dental epithelium fuse to form Hertwig's epithelial root sheath (HERS), which grows down to form the root, presumably by inducing the differentiation of odontoblasts. The role of the Dlx3 transcription factor in these processes is unknown, but the taurodontism observed in its absence suggests there is one. Again, the consistent lack of clinical and radiographic evidence of root defects in AI kindreds with *AMELX*, *ENAM*, *MMP20*, and *KLK4* mutations argues strongly against enamel matrix proteins playing a vital role in the differentiation of cementoblasts or root odontoblasts. Cementum forms after the root sheath disintegrates and its mineralization is necessary for attachment of the periodontal ligament. Alkaline phosphatase is an enzyme that is covalently bound to the outer membrane of cells expressing it. Cementum deposition in the primary teeth is sensitive to inhibition by pyrophosphate, possibly due to higher concentrations of enzymes that produce it (53).

Summary

Recent advances provide a better understanding of the regulatory networks and extracellular matrix molecules involved in tooth development, as well as the types of inherited defects that occur when the genes encoding these molecules are functionally altered by mutation. Early developmental processes shape the teeth and define the extracellular spaces that mineralize. Critical processes early in tooth formation are involved in a series of epithelial–mesenchymal interactions, and a loss of function of a critical component leads to an arrest of tooth development. Late processes in crown formation are devoted to secreting proteins and mineralizing the extracellular matrix. Loss of function mutations affecting crown formation translate into defects in the dentin and enamel matrices and result in inherited defects in mineralization. Enamel extracellular matrix molecules are not differentiation factors, but these proteins and their cleavage products are reabsorbed through endocytosis by fully differentiated cells and may provide information used to regulate secretory cycles and accommodate the dynamic needs of biomineralization. After the shape of the crown is fully defined, interactions involving HERS, cementoblasts, and odontoblast control the formation of tooth roots. Regulatory defects during root formation can lead to morphological defects, such as taurodontism, while mineralization defects can cause cementum agenesis and premature exfoliation of primary teeth.

Clinical utility and implications

Inherited diseases that manifest themselves primarily or exclusively as defects in the dentition pose a special challenge to dental practitioners, who are often the first health care providers to discover the pathology and make the original clinical diagnosis. Scientific advances are creating the opportunity as well as the expectation that a clinical diagnosis be expanded to include identification of the specific gene and mutation that causes the disease. Appropriate genetic tests are already available for some of the more common genetic diseases. The ordering of genetic tests by clinical dental practitioners is predicted to become the standard of care as three conditions are realized: 1) continued research completes the typically short list of candidate genes for each disorder, 2) genotype-phenotype correlations limit the list of probable candidate genes for a given patient, and 3) PCR-based genetic tests make mutational analyses practical and affordable. Accomplishing these objectives is a priority of current research funding agencies. Perhaps the most compelling argument for pursuing the goal of bringing genetic diagnoses into the scope of dental practice comes from the discovery that familial tooth agenesis caused by *AXIN2* mutations is closely linked to the development of colorectal cancer. Should not patients presenting with familial tooth agenesis be made aware of its potential association with cancer and have the mutation(s) underlying their disorder identified to learn if the risk applies to them?

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