Developmental Biology: Frontiers for Clinical Genetics

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Genetic basis for tooth malformations: from mice to men and back again


Teeth arise from sequential and reciprocal interactions between the oral epithelium and the cranial neural crest-derived mesenchyme. Their formation involves a precisely orchestrated series of molecular and morphogenetic events. Numerous regulatory genes that have been primarily found in organisms such as Drosophila, zebrafish, xenopus and mouse are associated with all stages of tooth formation (patterning, morphogenesis, cytodifferentiation and mineralization). Most of these genes belong to evolutionary conserved signaling pathways that regulate communication between epithelium and mesenchyme during embryonic development. These signaling molecules together with specific transcription factors constitute a unique molecular imprint for odontogenesis and contribute to the generation of teeth with various and function-specific shapes. Mutations in several genes involved in tooth formation cause developmental absence and/or defects of teeth in mice. In humans, the odontogenic molecular program is not as well known as that of mice. However, some insight can be obtained from the study of mutations in regulatory genes, which lead to tooth agenesis and/or the formation of defective dental tissues.

Conflict of interest

There is no existing conflict of interest.

Mechanisms of tooth development

Teeth are derived from cranial neural crest-derived mesenchyme (also called ectomesenchyme) and epithelium of the first branchial arch and a part of the frontonasal process (1–4). Irrespective of the generation (primary/permanent) and class of teeth (incisors, canines, premolars, and molars), odontogenesis proceeds in morphologically distinct stages (Fig. 1). Similar to the development of other organs that form as epithelial appendages (hairs, whiskers, nails, glands), tooth formation starts with epithelial thickenings at the sites of the future dental arches in the maxilla and mandible, which are called the dental placodes. Subsequent features of odontogenesis include the budding of the epithelium and the concomitant mesenchymal condensation, the continuous folding of the epithelium that is...
A series of sequential and reciprocal epithelial–mesenchymal interactions regulates all stages of odontogenesis, from tooth initiation to cytodifferentiation. A well-conserved molecular ‘dialog’ is used for the communication of epithelial and mesenchymal cells (2, 3, 6, 7). Signaling molecules control all steps of tooth formation by coordinating cell proliferation, differentiation, apoptosis, extracellular matrix synthesis and mineral deposition. The same molecules are repetitively used during the different stages of tooth development and are regulated according to a precise timing mechanism. The main molecules that are involved in tooth development belong to five signaling pathways: Notch, bone morphogenetic protein (BMP), fibroblast growth factor (FGF), sonic hedgehog (Shh) and Wnt. These signaling pathways involve numerous other molecules such as cell-surface receptors and transcription factors, which regulate gene expression (2, 6–8).

**Role of neural crest cells and oral epithelium in tooth initiation**

The first direct evidence of participation of neural crest cells in tooth formation was achieved following DiI injection into the midbrain and anterior hindbrain of rat embryos (9). This allowed the analysis of labeled crest cells within odontogenic regions of the first branchial arch. Genetic markers such as Wnt1 were also used to clearly show that dental mesenchyme in mice is derived from cranial neural crest cells (10).

Classical tissue recombination experiments between mouse oral epithelium and ectomesenchyme have
identified the oral epithelium as providing the instructive information for the initiation of tooth formation (11, 12). However, it is probable that the oral epithelium used in these experiments had already acquired a pre-pattern as a consequence of a prior interaction with cranial neural crest cells. Indeed, tooth-like structures were formed in mouse/chick chimeras, where the chick crest cells were replaced by mouse crest cells (13). These transplantation experiments clearly indicated that cranial neural crest cells also contain odontogenic potential and contribute equally with the oral epithelium to the initiation of tooth formation (14).

**Genetic basis for the dental field determination**

The territories where the teeth will grow in the oral epithelium as well as the tooth numbers are genetically determined from the very early stages of embryonic development. The transcription factor Pitx2 defines the oral epithelial area, where teeth will grow (15, 16). Deletion of Pitx2 results in the complete arrest of tooth development before placode formation (17, 18). Strong epithelial signals are needed to create dental placentas. Several signaling molecules have been implicated as activators (FGFs, Wnt) or inhibitors (BMPs) of placode formation (1, 2, 6, 7, 19). Molecules of the ectodysplasin (Eda) signaling are also involved in the formation and growth of the dental placentas in mice (20). Increased Eda signaling in transgenic mice contributes to larger than normal dental placentas and results in the development of extra teeth (21). In contrast, inactivation of Eda signaling in the Tabby mouse causes partial tooth agenesis and misshaped first molars. However, this tooth phenotype can be rescued after injection of Eda protein to pregnant Tabby mice (22). The most severe phenotype in mice is caused by p63 and Runx2 deletion, which results in the developmental arrest of all teeth (23, 24).

**Genetic basis for the position, number and shape of teeth**

The budding of the dental epithelium is followed by a mesenchymal condensation around the bud (Fig. 1). A strict molecular program, which is crucial for the continuation of tooth development, monitors these cellular events. During the bud stage, the odontogenic potential shifts from the dental epithelium to the condensing mesenchyme that can instruct any kind of epithelium to form tooth-specific structures (12). This transition is tightly regulated by interactions between the dental epithelial and mesenchymal tissues. Epithelial signals such as BMP, FGF, Shh and Wnt molecules may determine the display and fate of the ectomesenchyme for the generation of distinct tooth shapes/classes (1, 2, 6, 7, 25, 26). From all these molecules, BMP4 and FGF8 constitute essential early oral epithelial signals for the activation of specific genes in the underlying mesenchyme (2, 7, 25, 27). It has been suggested that various concentrations of these two signals could control tooth patterning; BMP4 directs the shape of incisors, while FGF8 directs the shape of molars (7, 25, 27). BMP4 induces in the ectomesenchyme the expression of Msx1 and Msx2, providing thus the spatial information for incisor patterning (1, 15, 16, 27). FGF8 induces in the ectomesenchyme the expression of Barx1, Dlx1, Dlx2, Lhx6 and Lhx7 genes, which are indispensable for molar morphogenesis (1, 15, 16). Members of each family of these homeobox genes may have compensatory functions. Indeed, the simultaneous inactivation of Msx1 and Msx2, or Dlx1 and Dlx2 results in arrest of tooth formation at the initiation stage (Fig. 1) (28, 29). Pax9 is expressed in the mesenchyme of both incisors and molars (30, 31). Isetl1 is expressed only in the incisor field of the oral epithelium (32). A regulatory loop exists between Isetl1 and BMP4 in the oral epithelium (32). Ectopic expression of Isetl1 in the molar field of the oral epithelium leads to downregulation of Barx1 expression in the mesenchyme, entailing the inhibition of molar development (32).

Thus, the complementary expression of the above genes defines territories associated with the formation of the various classes of teeth (1, 25) (Fig. 2). On the basis of the restricted and combinatorial expression domains of signaling molecules and transcription factors in the epithelium and ectomesenchyme of the first branchial arch, a ‘co-operative genetic interaction’ model has been proposed (25). Neural crest-derived cells, transcription factors and signaling molecules collectively contribute to the position, number and shape of teeth (25). In mice, mutations in genes encoding for several transcription factors such as Lef1, Msx1, Pax9 and Runx2 result in developmental arrest of all teeth at the bud stage (2, 7, 26) (Fig. 1).

**Genetic basis for the maxillary and mandibular dentition**

The molecular pathways that control tooth formation in the maxilla and mandible are not the same. Several genes such as Dlx are differentially expressed in the maxillary and mandibular processes (33), thus indicating a genetic difference between maxillary and mandibular tooth specification. Indeed, only the maxillary molars failed to develop in double mutants that lack both Dlx1 and Dlx2 (Dlx1; Dlx2-/-) (34). Pitx1 is another gene controlling mandibular identity. In the ectomesenchyme, Pitx1 is exclusively expressed in the proximal part of the developing mandible where molars will develop (35). In mice, Pitx1 deletion results in small misshaped mandibular molars (35). Similarly, deletion of the mesenchymal gene Activin B4A in mice leads to the selective loss of the incisors and mandibular molars, while the maxillary molars develop normally (36, 37).

**Genetic basis for the ameloblast fate**

Dental epithelial cells differentiate into ameloblasts, which form the hardest mineralized tissue of the body.
Fig. 2. Expression of transcription factors in the ectomesenchyme and epithelium of the nasal (np), maxillary (mx) and mandibular (md) processes during embryogenesis. (a) Neural crest-derived cells that migrate into the first branchial arch of an embryo are under the influence of signaling molecules and transcription factors (indicated by a variety of colors). Disposal of the various teeth on the dental axis is time-dependent, and combinations of different signaling molecules and transcription factors will contribute to a variety of tooth shapes/classes (incisors, canines, premolars and molars). Defects in the number of neural crest cells, signals (e.g. SHH) or transcription factors (e.g. MSX1) are responsible for misshaped teeth and/or tooth agenesis. (b) Expression of Barx1 in the first branchial arch mesenchyme of a mouse embryo. (c) Expression of Tbx1 in oral epithelium of a mouse embryo. oc, oral cavity.

The specification of these cells involves molecules of the Notch pathway (38, 39) and the Tbx1 transcription regulator (40, 41). Notch genes encode transmembrane receptors that participate in communication between neighboring cells (42). Notch receptors are activated by transmembrane ligands belonging to the Delta or Jagged families. In the developing teeth, Notch and Jagged and Delta are expressed in neighboring cell layers of the dental epithelium (38, 39, 43–45). Notch signaling has a pivotal role in the establishment of the tooth morphology and cytodifferentiation as Jagged2 mutant mice exhibit teeth with an abnormal shape and absence of the enamel matrix (39, 45).

Clinical and genetic findings have shown that Tbx1 also plays a significant role for the determination of dental epithelial cells to adopt the ameloblast fate. Patients with the DiGeorge syndrome, which is a TBX1 dependent disorder, exhibit hypodontia and enamel defects (46). In mice, Tbx1 deletion leads to hypoplastic incisors that lack enamel (40). FGF molecules affect both Tbx1 expression and proliferation of ameloblast progenitors in dental epithelium (41).

Human congenital tooth malformations

While there is an excellent agreement in morphologic stages of tooth development in mice and humans, knowledge on the molecular control of odontogenesis in humans is necessarily limited. However, some insight is given by experiments of nature, which lead to hereditary developmental malformations such as tooth agenesis or enamel dysplasias.

Tooth agenesis

The term tooth agenesis denotes a condition where deciduous (primary) and/or permanent teeth fail to develop. It is likewise referred to as hypodontia, but the same term is also used to characterize agenesis of up to six teeth (excluding the third molars) only (Fig. 3a). In contrast, agenesis of more than six teeth (Fig. 3b) or all teeth of a particular class (Fig. 3c) is referred to as oligodontia, and the term anodontia denotes the extremely rare condition, where all teeth fail to develop.

Tooth agenesis is the most frequent developmental malformation of the orofacial complex. However, prevalence ratios vary considerably between generations and classes of teeth and reveal some characteristic patterns. Thus, with a frequency of less than 1%, agenesis of primary teeth is rare (47, 48). Among the permanent teeth, absence of at least one third molar (prevalence 20–30%) is the most abundant.
Agenesis of the other permanent teeth is significantly more frequent in females than in males and varies between continents (49). As a rule, the teeth of a class which are formed last, most often fail to develop. Thus, when disregarding third molars, mandibular second premolars are missing most frequently (prevalence about 3%); followed by maxillary second incisors and maxillary second premolars (49). With increasing numbers of teeth missing in an individual patient, prevalence rates of tooth agenesis decrease markedly, and the frequency of oligodontia is only about 0.1–0.2% (49).

Most cases of tooth agenesis occur without developmental defects in other organs and are referred to as non-syndromic. However, missing teeth are also observed in association with other malformations, most noticeably with cleft lip with or without cleft palate (CLP). Interestingly, tooth agenesis outside the cleft area is also significantly higher than in the general population (50, 51). Even if hypodontia apparently is non-syndromic, it is commonly associated with a general reduction of crown sizes and a retardation of tooth formation (52–55). Thus, tooth agenesis could be regarded as an extreme tooth size reduction, which occurs below a certain critical threshold of odontogenic potential (51, 52).

Genetic defects so far could only be identified in severe forms of non-syndromic tooth agenesis. They affect the homeobox gene MSX1 (56–59), the paired-box gene PAX9 (26, 60–71), and AXIN2, the gene for an intracellular antagonist of Wnt signaling (72). All these three genes have been shown to be important regulators of early stages of tooth development in mice, particularly at the transition from the bud to the cap stage (50, 51). Therefore, mutations causing oligodontia in humans are assumed to result in an arrest of tooth development at the bud stage, when haploinsufficiency reduces gene dosage and, hence, tooth-forming potential below a critical level (51). However, contrary to what could be expected based on the purported role of MSX1, PAX9, and AXIN2 in tooth development, oligodontia caused by defects in these genes reveals typical, although partly overlapping and highly variable patterns of tooth agenesis (Fig. 3b,c). PAX9 and MSX1 mutations in humans do not affect all teeth of the same class (56–68). For example, mutations in the PAX9 gene affect mostly molars. Mutations in AXIN2 seem to cause a combination of MSX1 and PAX9 phenotypes. They affect exclusively the permanent dentition, but never the maxillary central incisors (51, 72). These, as well as canines and first molars are very stable teeth and seldom congenitally missing (73). The observation that the susceptibility for the consequences of a particular genetic defect varies between primary and permanent teeth as well as between classes and types of teeth raises some questions as to the universal validity of the concepts regarding the control of tooth formation. In this context, it should not be forgotten that the most common model for the study of odontogenesis, the mouse, possesses only one generation and two classes of teeth.

Genetic defects associated with syndromic forms of hypodontia mostly affect genes other than those causing non-syndromic oligodontia. An exception is MSX1, mutations of which can also result in Witkop syndrome, where defects of finger and toe nails accompany tooth agenesis (74). The most profound effects on skin and its appendages in combination with hypodontia and a reduction in tooth size is observed in some forms of ectodermal dysplasia, which are caused by genetic defects in the EDA pathway, i.e. the EDA, EDAR, EDARADD, IKKγ, NEMO, and p63 genes (50, 51, 75–78). Interestingly, mutations in EDA can also be responsible for non-syndromic hypodontia (79, 80). A striking phenotype of tooth agenesis characterized by the consistent, otherwise extremely rare absence of the maxillary central incisors is observed in Rieger syndrome, which is caused by defects in the homebox gene PITX2 (51, 77, 81).

Familial occurrence and concordance of tooth agenesis in twins suggest a significant genetic influence also in the milder forms of hypodontia, for example the common premolar-incisor agenesis (51, 82–85). If oligodontia is regarded as a consequence of a critical deficiency in gene dosage, it is conceivable that milder hypodontia results from DNA sequence variants, which have less severe effects on gene function. However, searches for polymorphisms in candidate genes responsible for oligodontia yielded inconsistent results (86–90). However, a significant role in hypodontia was shown for variants and haplotypes of TGFA (86, 91), IRF6 (92, 93), FGFR1 (92) as well as MMP1 and MMP20 (94). Thus, there does not seem to be a major hypodontia locus (51).

Amelogenesis imperfecta

The term amelogenesis imperfecta (AI) designates hereditary developmental malformations of tooth enamel. In a strict sense the definition includes only enamel dysplasias that occur in the absence of defects in other tissues. A now widely accepted nomenclature of AI relies on the mode of inheritance, the phenotype, and (if known) the molecular cause of the enamel defect (95). The phenotypic classification takes into account that enamel is formed in two major steps. In a first step, the secretory stage of amelogenesis, ameloblasts secrete an organic matrix in which hydroxyapatite crystals are loosely deposited. In a second step, most of the matrix proteins are degraded and resorbed by the ameloblasts, while the crystals grow in thickness until they come into contact with each other and the mineral density of enamel attains about 95% (96). Disturbances of the secretory stage result in the hypoplastic type of AI characterized by a quantitative deficiency of the enamel (Fig. 4a–f). The quantitative deficiency ranges from (virtual) aplasia, termed smooth hypoplastic AI (Fig. 4a,c,e), to vertical furrows (Fig. 4b), horizontal grooves, or pits (Fig. 4d,f), collectively referred to as rough (local) hypoplastic AI. Disturbances of the mineralization process result in the hypomineralized type of AI characterized by a qualitative deficiency, i.e.
Fig. 4. Smooth (a, c, e) and rough (b, d, f) hypoplastic forms of amelogenesis imperfecta. (a) Intraoral view of the maxillary dentition of a female patient showing the conical shape of the tooth crowns, which in the absence of enamel is determined by the dentin cores (crowns of the incisors had been constructed prosthodontically). Note the missing right second molar (arrow), the eruption of which is delayed in comparison with the contralateral tooth. (c) Intraoral radiograph from the brother of the patient shown in (a): No enamel can be recognized. (e) Backscattered electron micrograph of an upper third molar surgically removed from the patient shown in (a): In comparison with the enamel (E) of a healthy third molar (g), the mineral density of the enamel-like material of the patient’s tooth is essentially normal, but its thickness is only about 3–5%. (b) Frontal view of the incisors of a female patient showing vertical enamel furrows and streaks (arrows), as they occur as a result of X-chromosome inactivation (Lyonization). How these furrows and streaks arise is illustrated by a light micrograph from an incisor tooth germ (h) at the stage of enamel (E) and dentin (D) formation: X-chromosome inactivation is a random process taking place in the stem cells, which reside in the cervical loop (CL). The result of inactivation is propagated to all descendant daughter cells that differentiate in the vertical direction (arrow) and produce new inner enamel epithelial (IEE) cells to finally become enamel-forming cells, ameloblasts. As a consequence, clusters of ameloblasts arise along the circumference of the tooth germ, which carry an X chromosome with either a normal or defective AMELX gene and alternatively produce a normal or defective enamel matrix. (d) Frontal view of the incisors of a female patient showing enamel pits. (f) A backscattered electron micrograph of a maxillary premolar from the same patient reveals similar pits associated with regions of slightly hypomineralized enamel (arrow), which account for the slight yellow-brown discoloration. D, dentin, DP, dental papilla, OEE, outer enamel epithelium. Original magnifications (e) ×110, (f, g) ×50, (h) ×20.

enamel of about normal thickness, but reduced mineral and increased protein content (Fig. 5a–g). A moderate reduction in mineral density largely confined to the borders between the enamel prisms (Fig. 5e) leads to the hypomatured type of AI (Fig. 5a,c,e). The result of an even more severe mineral deficiency and protein retention is the hypocalcified form of AI (Fig. 5b,d,f). The majority of genetic defects responsible for AI have been identified in genes for enamel matrix proteins and enzymes required for the degradation of the enamel
matrix during the maturation stage. The gene for the most abundant enamel matrix protein, amelogenin, is located on the X- and Y-chromosomes, but in males about 90% of the protein is transcribed from AMELX, the copy on the X-chromosome (96, 97). Hence, mutations in AMELX cause X-linked AI which reveals diverse phenotypes, depending on the gender and the site of the mutation (96). In particular, hypoplastic forms of AI differ between sexes. While males usually present smooth hypoplastic AI, the phenotype in females is characterized by vertical furrows, which are due to X-chromosome inactivation (Fig. 4b) (98). Mutations in the signal peptide of AMELX resulting in failure of protein secretion as well as defects that
truncate the critical carboxy-terminus of amelogenin, cause smooth hypoplastic AI. In contrast, mutations in the amino-terminal region of AMELX which remove or alter proteinase cleavage sites, result in a hypomaturated AI type (97, 99).

While so far no defects responsible for AI could be detected in the AMBN gene encoding ameloblastin, several mutations were identified in the gene for enamelin (ENAM) located on chromosome 4q21 (97, 99–102).

They result in autosomal dominant or recessive smooth or rough hypoplastic AI. While the smooth hypoplastic phenotype resembles that seen with AMELX mutations, rough hypoplastic AI caused by defects in ENAM shows a peculiar phenotype characterized by horizontal grooves (103). It has been hypothesized that the enamel defects due to mutations in ENAM are dose dependent, smooth and rough hypoplastic phenotypes segregating as a recessive and dominant trait, respectively (100).

A further group of mutations causing AI affect the genes for enamelysin (MMP20) and kallikrein 4 (KLK4), both of which are tooth specific and important for proper enamel maturation (96, 99, 104, 105). In fact, all the identified genetic defects result in a loss of function of the enzymes and autosomal recessive AI of the (pigmented) hypomatured type. Rather surprisingly, several mutations causing a very similar AI phenotype were recently identified in WDR72, the gene for WD repeat-containing protein 72 (106, 107). It is indeed expressed in maturation-stage ameloblasts (107), but was previously not known to be involved in enamel formation.

Responsible mutations for the autosomal dominant hypocalcified AI, which is the third major form of AI and the most common in North America, have been identified only recently. Kim et al. (108) reported two nonsense mutations in FAM83H (family with sequence similarities 83 member H), which perfectly segregated with the disease. Identification of additional defects in the same gene (109–112) allowed genotype–phenotype correlations (113). All mutations, most of them nonsense mutations, occur in the last exon and considerably truncate the putative protein. It has been hypothesized that a short protein results in generalized hypocalcified AI, while a less severely truncated protein leads to a conspicuous attenuated phenotype characterized by hypocalcified enamel confined to the cervical part of the crowns (113). As the FAM83H gene is not expressed exclusively in teeth (108), a further question is why mutations cause AI without any apparent consequences in other tissues. It is speculated that enamel mineralization critically depends on high protein levels and that the truncated protein could exert a dominant negative effect (109, 113).

In summary, the distinct phenotypic forms of AI are genetically heterogeneous. Mutations in both AMELX and ENAM can result in either smooth or rough hypoplastic AI, and the hypomaturated type can be caused by genetic defects in AMELX, MMP20, KLK4, and WDR72. Only mutations in FAM83H seem to consistently result in hypocalcified AI, although with some variation in expressivity. However, overall less than half of the AI cases can be accounted for by defects in candidate genes known to date (114, 115).

Conclusion

Over the last years, a big effort has been made to understand the molecular and cellular mechanisms controlling tooth development and pathology. Much information on the genes that are important for human tooth formation has been revealed using the mouse model. However, very little is known on the generation of well-known human dental pathologies that are the consequence of aberrant cell differentiation and subsequent dental matrix formation. The complex genetic interactions leading to these human dental malformations can be only studied in detail in transgenic mice. Elucidating when and how signaling molecules and transcription factors dictate tooth initiation, morphology and mineralization will open new horizons to the dental discipline and will create new challenges. Novel genetic knowledge together with tissue engineering and stem cell approaches are probably to instruct development of novel therapies in dentistry (116, 117).

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References

Tooth genetics

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