Molecular Mechanisms of Tooth Development and Malformations

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Summary: Teeth develop as epidermal appendages from reciprocal inductive interactions between the epithelium and the mesenchyme. The morphological and molecular mechanisms defining tooth formation are highly conserved and resemble the morphogenesis of other epithelial organs such as lung, hair or kidney. Indeed, tooth development presents a powerful system for the understanding of more general questions of organogenesis. In recent years, the mechanisms underlying tooth formation and disease have been elucidated by the identification of causative genes in human malformation syndromes, the generation of genetically manipulated mice, and tissue recombination experiments. In the future, it is conceivable that molecular findings may lead to a regrouping of tooth malformations in genetic terms. In this paper we present an overview covering molecular concepts of tooth development and malformation conditions based on orthodontic findings, taking into account recent molecular and developmental insights.

Key words: amelogenesis imperfecta, dentinogenesis imperfecta, supernumerary teeth, hypodontia, tooth development

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INTRODUCTION

Teeth are unique structures that are exclusively located in the oral cavity. They consist of several cell types, including ameloblasts, odontoblasts and cementoblasts producing enamel, dentin and cement, respectively. Teeth develop from reciprocal interactions between epithelial and mesenchymal tissue, leading to the formation of epithelial buds and the subsequent morphogenesis and differentiation. More than 200 genes have been found to be involved in tooth development (http://bite-it.helsinki.fi), acting in conserved pathways. Considerable parallels have been found for the development of teeth and other organs such as lung, hair or kidney, indicating that teeth present a powerful system for the understanding of more general questions of organogenesis.

The human set of teeth consists of two incisors, one canine, two premolars and three molars on each side of the jaws. Mice have only one incisor on each side of the jaw, and lack canines and premolars, which are replaced by a space without teeth, the diastema. In addition, mice only have one set of teeth, whereas humans exhibit a set of deciduous and permanent teeth. Despite these differences in tooth number and shape the embryonal mechanisms underlying tooth formation show considerable similarities between mice and human, making the mouse an attractive organism to study the development of mammalian teeth.

TOOTH DEVELOPMENT AND ITS MOLECULAR BASIS

Teeth develop from a series of reciprocal interactions between the oral epithelium and the underlying cranial neural-crest-derived (ecto-)mesenchyme on the oral surfaces of the mandibular, maxillary and frontonasal process. During development of mammalian teeth patterning of the tooth region is followed by tooth induction,

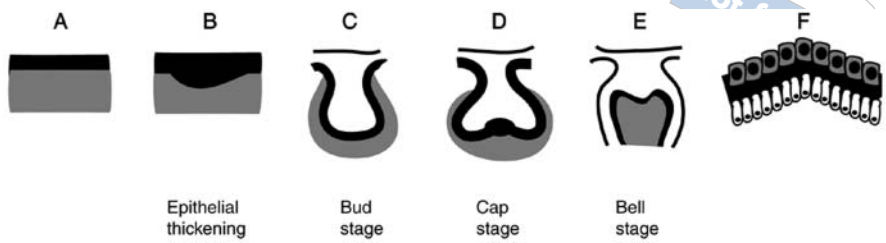

Fig. 1 Stages of molar tooth development. Sequential inductive interactions between the epithelium and the mesenchyme regulate tooth morphogenesis and cell differentiation. The epithelium is represented in black; the mesenchyme is presented in grey. A. and B. Local thickening of the oral epithelium indicates signs of tooth initiation. C. Budding of the dental epithelium leads to induction of mesenchymal cell condensations around the tooth germs. D. At cap stage the epithelium undergoes further folding and invaginates leading to the formation of the mesenchymal dental papilla and the enamel knot (EK). E. The cusp pattern in molars forms by continuous folding of the epithelium. Ameloblast and odontoblast precursors form at bell stage. F. Epithelial ameloblasts and mesenchymal odontoblasts terminally differentiate during advanced bell stage at the tissue interface, beginning at the tips of the future cusps. They deposit enamel and dentin, respectively (adapted from Thesleff and Nieminen, 1996).

morphogenesis and differentiation leading to a defined identity and shape (Fig. 1; for review, see Depew et al, 2002). Subsequently the cells from the stomodeal ectoderm give rise to ameloblasts, and cranial neural-crest-derived (ecto-)mesenchyme cells lead to the formation of odontoblasts and cementoblasts of mammalian teeth. Interestingly, the same molecules are involved during different stages of tooth development.

Patterning of Dentition

During development distinct types of teeth develop in different positions from an apparently uniform layer of oral ectoderm and the underlying (ecto-)mesenchyme. The molecular mechanisms that provide a positional code for morphogenesis of teeth is based on a code of homeobox genes expressed in (ecto-)mesenchymal cells prior to E11, which is referred to as odontogenic homeobox code (Sharpe, 1995; Thomas and Sharpe, 1998) (Fig. 2). *Msx1* and *Msx2* are expressed in the midline (ecto-)mesenchyme before the initiation of tooth germs in regions where incisors will form, whereas the expression domains of *Dlx1* and *Dlx2* are confined to the (ecto-)mesenchyme, where molars will develop (MacKenzie et al, 1991). The expression of *Barx1* corresponds to the subset of *Dlx1/2* expressing cells that will give rise to molars.

The homeobox model initially based on expression analysis was further supported by evidence from loss of function mouse models including the *Dlx1/2*$^{-/-}$ mice with a disturbed development of maxillary molar teeth, but normal incisors as predicted from the model (Qiu et al, 1995). Normal development of mandibular molars in these mice can be attributed to functional redundancy from other *Dlx* genes as *Dlx5* and *Dlx6* expressed in the mesenchyme. *Msx1/2*$^{-/-}$ mice exhibit arrest of all teeth at the epithelial thickening stage, indicating their role in early bud formation and possibly incisor patterning based on the homeobox code (Satokata et al, 2000). The code model was further supported by misexpressing *Barx1* in distal (ecto-)mesenchyme cells, resulting in incisor tooth germs developing as molars (Tucker et al, 1998b).

Initiation of Dentition

Tooth development is initiated when the oral ectoderm undergoes thickening at positions where future teeth will form at E9-11. The dental lamina, a thickened epithelial stripe, is formed and marks the future dental arch. Recombination experiments using epithelium and mesenchyme before E10.5 indicate the instructive influence of the ectoderm, whereas at later stages the odontogenic potential is switched to the mesenchyme (Lumsden, 1988; Mina and Kollar, 1987). Odontogenic neural crest cells are originally derived from the cranial neural crest, migrating and populating the pharyngeal arches. Recombination experiments using oral epithelium and nonodontogenic neural crest-derived mesenchyme from E9 and E11 embryos result in formation of normal teeth (Mina and Kollar, 1987).

Several factors from conserved signalling pathways have been implicated in early tooth induction. *Bmp4* is first detectable from E10 to E11.5 in the distal midline ectoderm (Vainio et al, 1993). At E11.5 *Bmp4* expression is shifted from the ectoderm to the (ecto-) mesenchyme. *Bmp2* is coexpressed with *Bmp4* at E11, but is not expressed in the mesenchyme later on (Tucker et al, 1998a). *Shh* is expressed in the presumptive dental ectoderm at E11 and is another candidate for tooth initiation. Implantation of Shh-soaked beads in the oral ectoderm leads to local epithelial cell proliferations and the formation of invaginations reminiscent of tooth buds, but not to the development of teeth (Hardcastle et al, 1998). This has suggested a role for *Shh* in stimulation of epithelial cell proliferation and tooth initiation. *Lef1*,

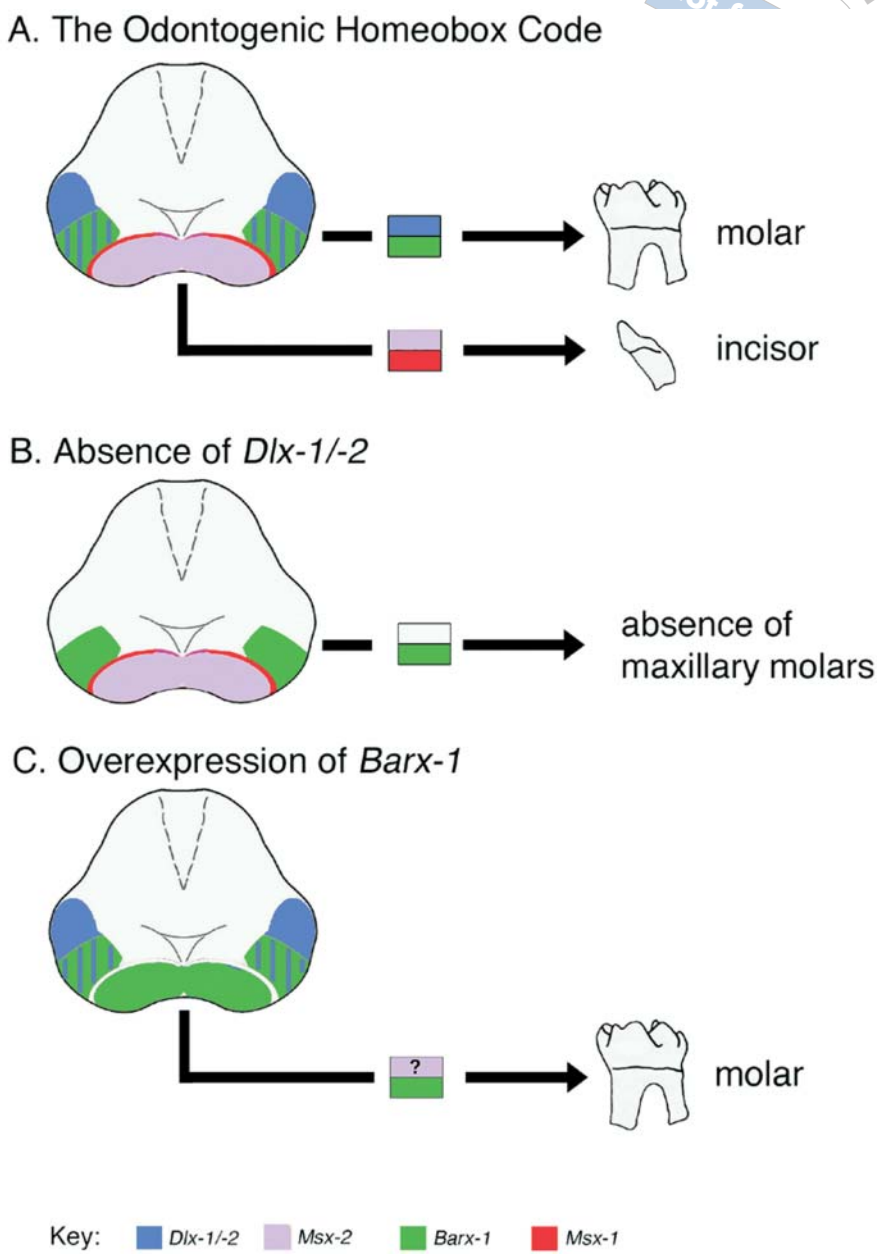

Fig. 2 Odontogenic homeobox code. A. The expression of homeobox genes provides a positional code for morphogenesis of the teeth. Expression domains of Msx1, Msx2 coincide with regions, where incisors will form. Dlx1, Dlx2 are expressed where molars will develop. The expression of Barx1 overlaps with the expression domains between Msx and Dlx. B. In Dlx-1/2$^{-/-}$ mice maxillary molars are absent. C. Overexpression of Barx1 in (ecto-)mesenchymal cells results in incisor tooth germs developing as molars.

a nuclear mediator of Wnt signalling, is another factor involved in tooth initiation. *Lef1* is expressed in dental epithelial thickenings and is shifted to the condensing mesenchyme, when the bud forms. In *Lef1*$^{-/-}$ mice dental development is arrested at bud stage. Moreover, using tissue recombination *Lef1* has been shown to be important in the dental epithelium prior to bud formation (van Genderen et al, 1994).

Bud to Cap Transition

After thickening, the epithelium of the dental lamina starts to form tooth buds and induces mesenchymal cell condensations around the tooth germs (bud stage). The bud stage marks a shift of inductive potential from the tooth epithelium to the mesenchyme, accompanied by a shift of expression of signals as *Bmp's* and *Fgf's* from the epithelium to the mesenchyme. Budding of the tooth is followed by the cap stage, when the tip of the epithelial tooth bud bends into a cap-like structure surrounding the condensed mesenchyme, which is referred to as dental papilla. The morphological differences between tooth germs that give rise to different types of teeth become apparent during the transition from bud to cap stage.

A number of knock out mice, exhibiting an arrested tooth development at the bud stage, have led to particular interest in the transition between the two stages. *Msx1* and *Bmp4* are expressed in mesenchymal cells condensing around the tooth buds and represent interesting candidates for the development of tooth shape (Chen et al, 1996). *Msx1*$^{-/-}$ mice exhibit an arrested tooth development at the bud stage (Bei and Maas, 1998) and can be rescued by addition of exogenous Bmp4 (Chen et al, 1996). Loss of *Shh* at different stages of tooth development has led to the identification that *Shh* signalling is required at different time points during mammalian tooth development. Blocking of Shh using neutralizing antibodies indicates that Shh is required for the formation of tooth buds at E11-12 and at E13 for tooth morphogenesis. If *Shh* activity is removed from the oral epithelium using a conditional knock out mouse model, molar tooth morphogenesis is disrupted with normal cytodifferentiation seen, indicating the importance of *Shh* during cap stage (Dassule et al, 2000). *Pax9* is another homeobox gene with an important role in bud-to-cap transition. *Pax9* is expressed in the mesenchyme of the bud, and in *Pax9*$^{-/-}$ mutants all teeth are arrested at the bud stage (Peters et al, 1998). *Activin-βA* is coexpressed with *Pax9* in normal development, but its expression is not altered in *Pax9*$^{-/-}$ mice, indicating that *Pax9* and *Activin-βA* function independently from each other (Matzuk et al, 1995; Ferguson et al, 1998).

Differentiation

Continuous growth and folding of the epithelium subsequently leads to the formation of the tooth crown in bell stage. Tooth morphogenesis is accompanied by differentiation of odontoblasts from the mesenchyme and ameloblasts from the epithelium. During the cap and bell stage a new signalling centre, the enamel knot (EK), is formed at the tip of the tooth bud, where it controls cusp morphogenesis. The EK consists of transient clusters of epithelial cells that can be seen in sections of molar cap tooth germs. For EK many parallels to other developmental signalling centres are obvious, such as the zone of polarising activity (ZPA) in the limb bud or the notochord of the floor plate. A number of genes from different signal transduction cascades are expressed in the EK in a highly dynamic spatial and temporal expression pattern. These include *Bmp2*, *Bmp4*, *Bmp7*, *Fgf9*, *Wnt10b* and *Shh* that regulate cell proliferation and apoptosis (Thesleff and Sharpe, 1997). Molars exhibit secondary EKs at the tips of forming cusps in the bell stage that are not present in incisors and are responsible for formation of their multicuspid shape.

During the advanced bell stage, epithelial ameloblasts and mesenchymal odontoblasts terminally differentiate at the interface of the tissue starting from the tips of the future cusps. Ameloblasts and odontoblasts deposit organic matrices, enamel and dentin, respectively.

DENTAL MALFORMATIONS

Congenital tooth defects may affect position, shape, size, structure, colour, root or eruption of teeth and are found in an isolated form or in association with a syndrome. Congenital tooth malformations are individually rare, however they are of clinical relevance because of their overall frequency and severity. Due to their related developmental origin syndromic dental anomalies may be associated with other defects in craniofacial developmental or of epithelial appendages such as hair, nail or sweat glands. The recent identification of genes responsible for congenital tooth defects has elucidated some of the molecular mechanisms underlying normal and pathologic tooth development. With respect to this, mouse genetics, transgenic technology and tissue transplantation experiments have also helped to increase the understanding of the developmental processes underlying tooth development and dysmorphogenesis.

Anodontia, Hypodontia, Oligodontia

Anodontia describes the congenital absence of all teeth. Hypodontia or oligodontia are used as synonyms that characterize a condition of having fewer than the normal complement of teeth, either congenital or acquired. Anodontia and hypodontia/oligodontia may involve both the deciduous and the permanent dentition, or only teeth of the permanent dentition. The most frequently affected teeth in hypodontia are third molars, followed by upper lateral incisors and/or lower second premolars (Symons et al, 1993).

Hypodontia may appear as an isolated trait or in association with other developmental defects and is often associated with defects in other epithelially derived organs and with craniofacial malformations. In conditions with cleft lip/palate, hypodontia and microdontia are usually closely localized within the region of the cleft. In unilateral clefts hypodontia may also be seen on the contralateral side. To date, more than 50 syndromes have been described that exhibit hypodontia or oligodontia (Table 1).

Isolated autosomal dominant Hypodontia (HYD; MIM 106600) in humans is caused by missense muta-

Table 1 Overview of dental anomalies with corresponding tooth phenotype and molecular defect

Dental anomaly	Disorder	Genomic Locus	Gene
Tooth number			
Hypodontia/Oligodontia	Hypohidrotic ectodermal dysplasia	Xq12-q13.1	*EDA*
		2q11-q13	*EDAR*
		1q42.2-q43	*EDARADD*
	EEC, ADULT, LMS	3q27	*P63*
	Incontinentia pigmenti	Xq28	*NEMO*
	OFD I	Xp22.3-p22.2	*OFD1*
	Oligodontia	4p16.1	*MSX1*
		14q12-q13	*PAX9*
	Axenfeld-Rieger syndrome	4q25-q26	*PITX2*
		16q24.3	*FOXC2*
		13q14	
Supernumerary teeth	CCD	6p21	*RUNX2*
	OFD I	Xp22.3-p22.2	*OFD1*
	Opitz syndrome	Xp22	*MID1*
Tooth shape			
Taurodontism	TDO	17q21-q22	*DLX3*
Tooth structure			
Amelogenesis imperfecta	AIH1	Xp22.3-p22.1	*AMELX*
	AIH2	4q21	*ENAM*
	LADD		
	ODDD	6q21-q23.2	*CX43*
	OFD I	Xp22.3-p22.2	*OFD1*
	TDO	17q21-q22	*DLX3*
Dentinogenesis imperfecta	DGI-II, DTDP2	4q21.3	*DSPP*

Abbreviations: ADULT – Acro-dermato-ungual-lacrimal-tooth syndrome; **AIH –** Amelogenesis imperfecta; **CCD –** Cleidocranial dysplasia; **DGI –**Dentinogenesis imperfecta; **DTDP –** dentin dysplasia; **EEC –** Ectrodactyly ectodermal dysplasia, and cleft lip/palate syndrome; **LADD –** Lacrimoauriculodentodigital syndrome; **LMS –** Limb-mammary syndrome; **ODDD –** occulodental dysplasia; **OFD –** Orofacio-digital syndrome; **TDO –** Trichodentoosseous syndrome; **TRPS –** Trichorhinophalangeal syndrome

tions in *MSX1* (Vastardis et al, 1996). The mutations are located in the homeodomain likely to affect *MSX1* interactions. *Msx1* is expressed in the dental mesenchyme and regulates the expression of *Bmp4* and *Fgf3*. *Msx1*$^{-/-}$ mice exhibit multiple craniofacial malformations beside an arrested tooth development at bud stage (Satokata and Maas, 1994). The fact that *Bmp4* can rescue the *Msx1* tooth phenotype in culture indicates the molecular hierarchy and rescue by introduction of a downstream target (Bei et al, 2000).

Another gene identified as associated with isolated oligodontia is *PAX9*. Frameshift mutations resulting in an alteration of the paired domain of *PAX9* have been shown to be associated with autosomal dominant Oligodontia (Stockton et al, 2000). *Pax9*$^{-/-}$ mice exhibit an arrest of tooth development at the bud stage, accompanied by a defective development of pharyngeal pouch derivatives, craniofacial and limb anomalies (Peters et al, 1998).

Hypohidrotic Ectodermal Dysplasia

Hypohidrotic ectodermal dysplasia (HED) exists as an X-linked (ED1; MIM 305100) autosomal recessive and dominant form (MIM 129500) and is characterized by abnormal or missing teeth, missing or poorly developed hair, lack of sweat glands and sometimes nail dystrophy (Fig. 3). Dental findings include hypodontia to anodontia, and conical shape of the anterior teeth is a hallmark of HED. There is a delay in tooth eruption, and the height of the alveolar processes is reduced due to hypodontia. The mucous membranes of the mouth are less moistened because of a decrease in salivation. Craniofacial characteristics include a prominent forehead, a small nose, and prominent lips.

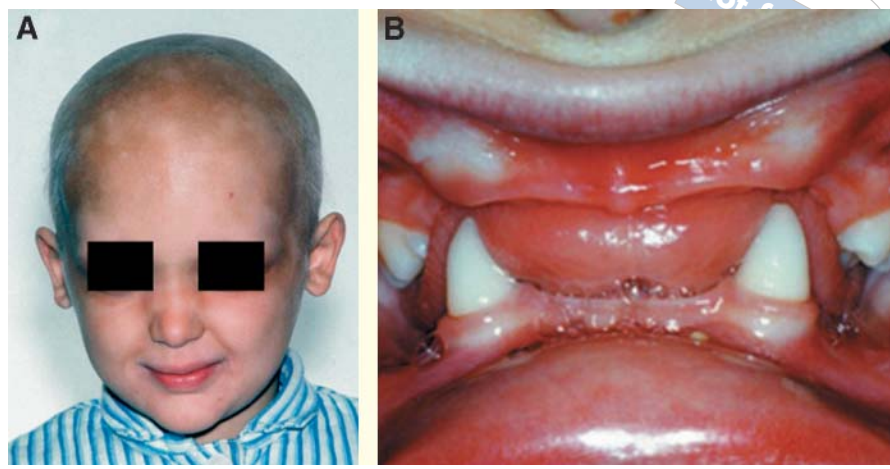

Fig. 3 Hypohidrotic Ectodermal Dysplasia (HED). A. Patient with HED showing loss of hair, thin skin and pigmentation defects. B. Hypodontia in HED with characteristic conical shape of the anterior teeth.

Mutations in *ectodysplasin* (EDA), a unique member of the TNF superfamily, have been shown to be responsible for X-linked HED (Kere et al, 1996) and have also been identified in the corresponding mouse mutant *Tabby (Tb)* (Ferguson et al, 1997). Positional cloning of another mouse mutant, the *downless (dl)* mutant, revealed mutations in a death domain containing TNF receptor, named *EDAR* (Headon and Overbeek, 1999) that binds the *EDA-A1* isoform. Mutations in the corresponding human *EDAR* gene lead to the autosomal recessive and dominant form of HED (Monreal et al, 1999). A third mouse mutant *crinkled (cr)* and a large family with HED, but without mutations in *EDA* and *EDAR* led to the discovery of an adaptor molecule named *EDARADD* that interacts with the receptor and links it to the downstream signalling pathway (Headon et al, 2001; Yan et al, 2002).

In the developing tooth *Eda* expression is found in the outer, but not in the inner enamel epithelium, and *Edar* is expressed in the EK. In the tooth, Eda signalling is mediated by NF-κB and mutations in components of the NF-κB pathway such as *Ikkα*, result in a molar cusp phenotype similar to *Tabby, downless*, etc. (Ohazama et al, 2004). Whereas *Tabby* mice exhibit a smaller EK with a normal shape (Pispa et al, 1999), in *dl* the EK has a normal size but the structure of the EK cells is altered (Tucker et al, 1998b). The molecular and morphological characteristics of these mouse mutants indicate the importance of the EK in cusp formation.

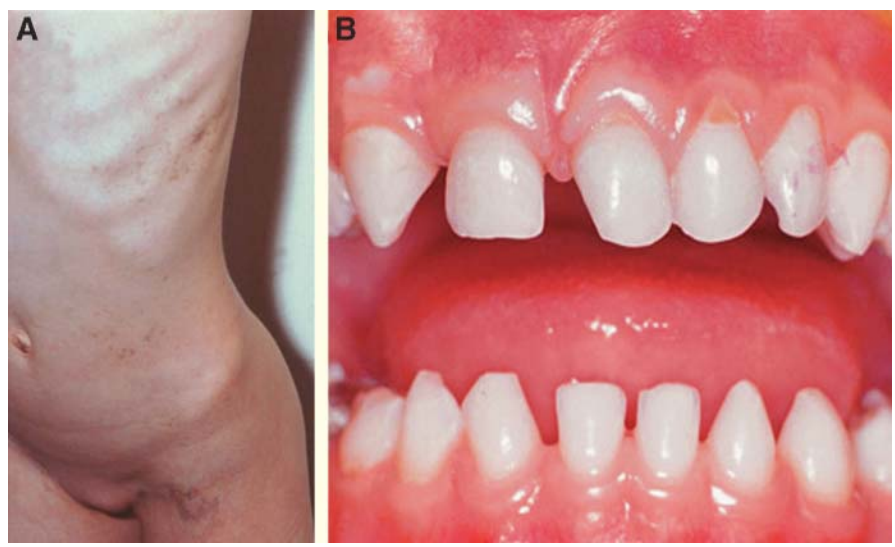

Fig. 4 Incontinentia pigmenti. A. The skin of a patient with incontinentia pigmenti (IP) shows vesiculation, verrucous changes, atrophy, and irregular pigmentation, particularly located at the trunk and extremities. B. Deciduous teeth with hypodontia in a patient with IP, exhibiting irregular teeth with cusp anomalies.

Incontinentia pigmenti

Incontinentia pigmenti (IP; MIM 308300) is an X-linked dominant disorder typically seen in females. Whereas for men IP is lethal, affected women survive due to dizygosity for the X-chromosome and selective proliferation advantages of the normal X chromosome. Skewed X-inactivation of the mutant X-chromosome is often used to confirm the diagnosis. IP is characterized by skin pigmentation abnormalities (Fig. 4) that begin with linear vesicular skin lesions, followed by a verrucous eruption and lead to hyperpigmented, marble-cake-like swirls which may fade in adulthood. The tooth phenotype is characterized by hypodontia in both deciduous and permanent teeth. Crowns have a conical shape, dentition is delayed and the teeth are malpositioned. Retinal detachment and central nervous disturbances may present in IP, other manifestations include hair loss and nail dystrophy. Diagnostic difficulties can arise from the multisystemic nature of the disease with significant expression variability.

IP is caused by mutations in the *NEMO (IKKγ)* gene that in 85% of patients exhibits an identical deletion, removing a large portion of the gene (Smahi et al, 2000). *NEMO (IKKγ)* is indispensable for *NF-κK* activation, and male *Nemo*$^{-/-}$ mice die during midgestation embryogenesis from liver apoptosis, similar to *NF-κK (p65)*$^{-/-}$ and *Ikk-β*$^{-/-}$ mice (Schmidt-Supprian et al, 2000). Female *Nemo*$^{-/-}$ mice exhibit strong overlap with the human IP phenotype, exhibiting skin lesions, incontinence of melanin, granulocyte infiltration and detachment of keratinocytes in the epidermis.

Axenfeld-Rieger Syndrome

Axenfeld-Rieger (AR) syndrome is an autosomal dominant disorder characterized by eye abnormalities of the anterior eye chamber including hypoplasia of the iris with aberrant synechiae and mesenchymal tissue filling in the angle of the anterior chamber. Orocraniofacial findings in patients with AR include hypoplasia of the premaxillary and maxillary region with a thin upper lip and everted lower lip and cleft palate. Patients with AR exhibit hypodontia or anodontia, manifesting as loss of deciduous and permanent upper molars and missing second premolars. Lower incisors and canines have a conical shape.

Frameshift and missense mutations in the homeobox transcription factor *PITX2* (Semina et al, 1996) and the forkhead domain gene *FOXC1* (Nishimura et al, 1998) are responsible for Rieger anomaly. Linkage analysis indicates another locus on chromosome 13q14 for RIEG1 (Phillips et al, 1996). *Pitx2* is a member of the paired bicoid family of homeodomain transcription factors. During odontogenesis *Pitx2* is coexpressed as a transactivator of procollagen lysyl hydroxylase *PLOD* that is mutated in Ehlers Danlos syndrome suggesting that the ocular and dental malformations observed in RIEG1 may be related to Ehlers-Danlos syndrome (Hjalt et al, 2001). *Pitx2* expression in the mandibular epithelium is defined by *Bmp4* and *Fgf8* acting in an inhibitory or activating fashion during odontogenesis indicating that the dental manifestations of RIEG1 are linked to an abnormal expression of *Bmp* and *Fgf* signals (St Amand et al, 2000). *Pitx2*$^{-/-}$ mice are lethal and about 10% of heterozygous mice display a reduced phenotype of human RIEG1 (Lin et al, 1999).

FOXC1 belongs to the family of forkhead transcription factors characterized by a winged helix structure. During development *FoxC1* has been shown to be expressed nearly ubiquitously, with high expression in the heart, muscle, liver and kidney (Pierrou et al, 1994). *Foxc1*$^{-/-}$ mice die around birth and exhibit a skeletal phenotype and abnormalities of the anterior occular chamber (Kume et al, 1998).

Oro-facial-digital Syndrome Type I (OFD I)

Oro-facial-digital syndrome type I (OFD I; MIM 311200) is transmitted as an X-linked dominant condition with lethality in males and is characterized by oral, craniofacial and digital malformations (Fig. 5). The oral features include a midline cleft of the upper lip, multiple hyperplastic oral frenula, clefting of the maxillary alveolar ridge into an anterior portion containing the canine teeth and two posterior segments, and a lobulated tongue with hamartomata. Lateral incisors are absent and the present anterior teeth are abnormal. In addition, dental findings may present as supernumerary upper incisors, canines and premolars, hypodontia of lower incisors, enamel hypoplasia and caries. Other craniofacial findings include telecanthus, hypoplasia of alar cartilages, and milia of upper face and ears in infancy. Asymmetric shortening of digits with variable degrees of syndactyly, clinodactyly and/or polydactyly are present. Mental retardation is seen in about half of the patients, and absence of the corpus callosum and heterotopia iridis occurs. Mutations in the *OFD1* gene, formerly named *Cxorf5/71-7a*, were found in one family and in a number of sporadic cases (Ferrante et al, 2001). During development *Ofd1* is expressed in various craniofacial structures such as the oral and nasal cavities, the tongue, the gum, developing tooth buds, Rathke's pouch, the central nervous system, the retina and the inner ear.

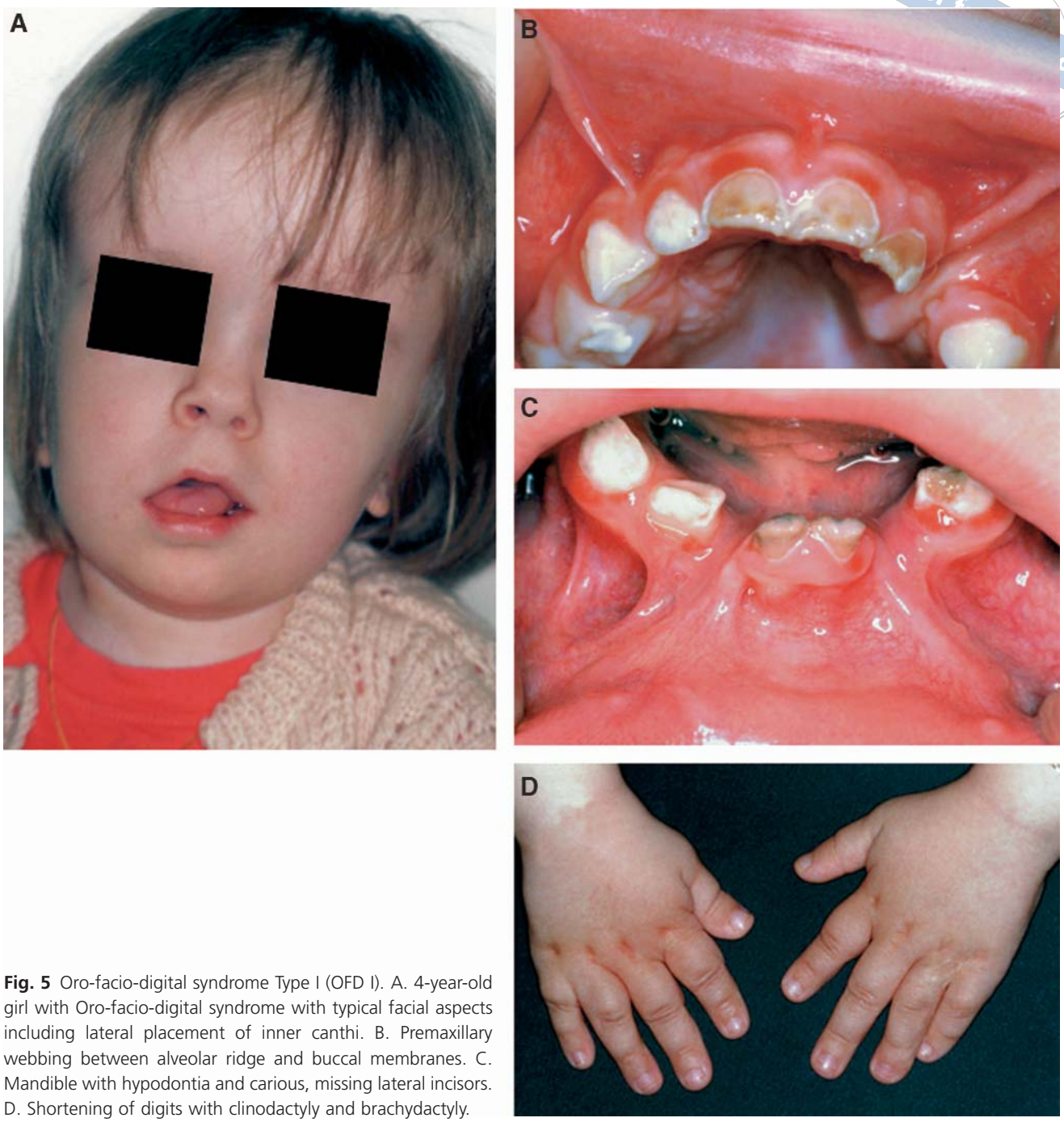

Fig. 5 Oro-facio-digital syndrome Type I (OFD I). A. 4-year-old girl with Oro-facio-digital syndrome with typical facial aspects including lateral placement of inner canthi. B. Premaxillary webbing between alveolar ridge and buccal membranes. C. Mandible with hypodontia and carious, missing lateral incisors. D. Shortening of digits with clinodactyly and brachydactyly.

Ectrodactyly Ectodermal Dysplasia Cleft Lip/ Palate Syndrome (EEC Syndrome) and Other P63 Associated Anomalies

EEC syndrome (MIM 129900) is an autosomal dominant disorder characterized by the triad ectrodactyly, ectodermal dysplasia and cleft lip/palate. Ectrodactyly (split-hand/foot malformations) occurs in a large variability, ranging from near normal to severe malformations (Fig. 6). The ectodermal features involve hair, teeth and nails: the hair is sparse, fair and dry, and the eyebrows and lashes are often absent; the teeth are small, and oligodontia/anodontia is present; and the nails are thin, brittle and ridged. Besides the triad defining the syndrome, further features include lacrimal

duct anomalies, urogenital malformations, conductive hearing loss, facial dysmorphism and mental retardation.

Heterozygous mutations in *P63* have been shown to be responsible for EEC syndrome predicted to lead to a dominant negative or gain of function mechanism rather than loss of function (Celli et al, 1999). *P63* is highly expressed in embryonic ectoderm and the basal regenerative layers of many epithelial tissues in the adult. *P63*$^{-/-}$ mice die at birth, lacking teeth, limbs, epidermis, prostate, breast and urothelial tissues, probably due to loss of the stem cells required for these tissues (Yang et al, 1999). Subsequently five other phenotypically related diseases have been mapped to human chromosome 3q27 and mutations in *P63* have been found in: Ankyloblepharon-ectodermal defects-cleft lip/palate syndrome (AEC; MIM 106260; McGrath et al, 2001); Limb-mammary syndrome (LMS; MIM 603543); Acro-dermato-ungual-lacrimal-tooth syndrome (ADULT; MIM 103285; Duijf et al, 2002); Split-hand/foot malformation type 4 (SHFM4; MIM 605289; Ianakiev et al, 2000); and Rapp-Hodgkin syndrome (RHS; MIM 129400; Kantaputra et al, 2003). The mutations for EEC and AEC syndrome are clustered in the DNA binding domain and SAM domain of the gene, respectively (for review, see van Bokhoven and McKeon, 2002). *P63* expression in the embryonic ectoderm and the basal layers of many epithelial tissues suggests a common pathogenetic mechanism for the derived developmental anomalies. The embryonal mechanisms for most of these alterations seem to be based on a common mechanism, as epithelial mesenchymal interactions during embryonal development take place in most of these organs and structures. Given that *P63* plays a central role in the epithelial stem cell fate, a major goal of future research will be to understand which genetic programs are used by the *P63* pathway.

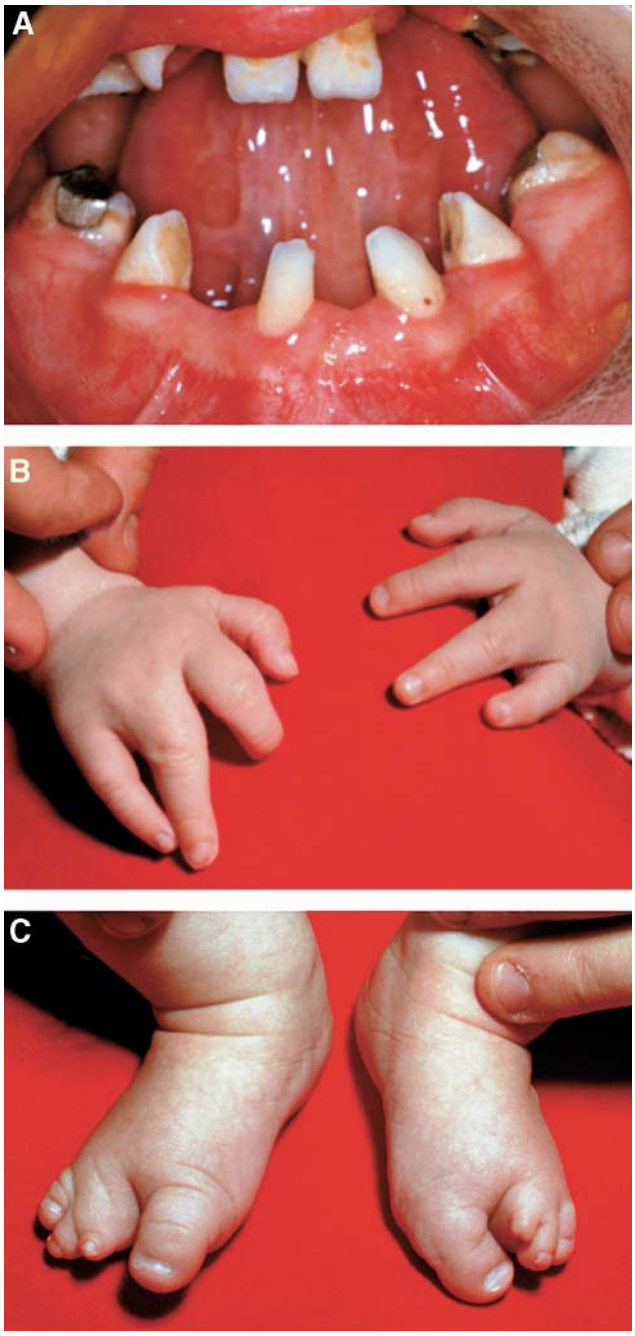

Fig. 6 Ectrodactyly Ectodermal Dysplasia Cleft Lip/Palate (EEC) Syndrome. A. Oligodontia in a patient with EEC syndrome. B. The patient's hands show oligodactyly and brachydactyly. C. Feet of the same patient exhibit the typical lobster claw with a missing second digit and nail dystrophy.

Supernumerary Teeth and Macrodontia

Supernumerary teeth are far less frequent than hypodontia and are mainly found within permanent dentition. Most frequently, premolars and lateral incisors are affected. Supernumerary teeth may present with normal or altered tooth shape. The mesiodens of the maxilla is a typical example for a dysmorphic supernumerary tooth.

Macrodontic teeth are frequently found closely located to duplicated or triplicated teeth, and macrodontia can be interpreted as a microfeature of a supernumerary tooth. In some cases incisors acquire shapes resembling premolars or even molars referred to as (pre-)molarisation. Supernumerary teeth are present in: Cleidocranial dysplasia (CCD; MIM 119600); Opitz G/

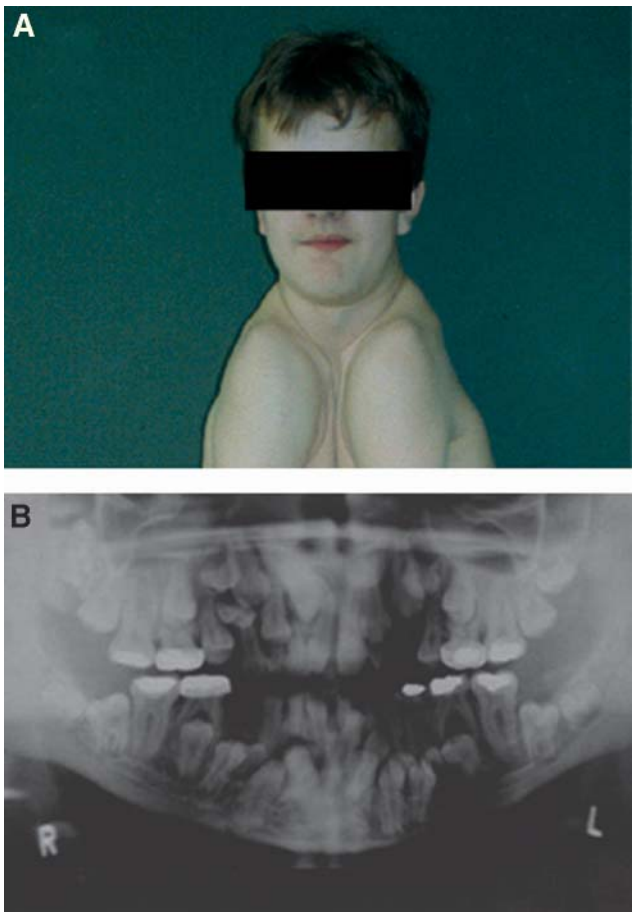

Fig. 7 Cleidocranial Dysplasia (CCD). A. Patient with CCD displaying characteristic hypoplastic clavicles. B. Orthopantomogram of patient with CCD showing late eruption of teeth and supernumerary teeth with aplasia and malformed roots, retention cysts and enhanced caries.

BBB syndrome (OS; MIM 300000); Oro-facio-digital syndrome (OFD1; MIM 311200); and Trichorhinophalangeal syndrome type I (TRPS1; MIM 190350).

Cleidocranial dysplasia

Cleidocranial dysplasia (CCD; MIM 119600) is an autosomal dominant disorder characterized by patent fontanels, late closure of cranial sutures with Wormian bones, dentition anomalies, absent or rudimentary clavicles, short stature and minor limb defects. Patients exhibit brachycephaly with bossing of frontal fontanels, calvarial thickening and incomplete development of accessory sinuses and mastoid air cells. Other craniofacial findings include midface hypoplasia, a low nasal bridge, a narrow high arched palate and hypertelorism. Dentition anomalies comprise late eruption, particularly of permanent teeth, abnormalities of shape and number including malformation of roots, retention cysts, enamel hypoplasia and supernumerary teeth (Fig. 7). Considerable phenotypic variability has been reported for CCD, ranging solely from dentition problems to individuals with generalized features of osteoporosis (Otto et al, 2002).

CCD is caused by heterozygous mutations in *RUNX2* (Lee et al, 1997; Mundlos et al, 1997), formerly known as *CBFA1*, *PEBP2-alphaA*, *AML3*, *NMP-2* and *til-1*. A genotype-phenotype correlation has been established between the height of CCD patients and the transactivation potential of *RUNX2* mutations as well as the height of patients and the number of supernumerary teeth, indicating a gene dosage effect for *RUNX2* mutations (Otto et al, 2002). During early odontogenesis *Runx2* is expressed in a thin layer of mesenchymal cells underlying the dental epithelium and later on during dentinogenesis in mesenchymal condensations of teeth (Yamashiro et al, 2002). Eventually, *Runx2* is downregulated in differentiated odontoblasts, but is expressed in ameloblasts during enamel formation (D'Souza et al, 1999). Therefore it is not surprising that *ameloblastin*, a tooth specific extracellular matrix protein involved in enamel crystal formation, is a target of *Runx2* (Dhamija and Krebsbach, 2001). In addition, the promoter of *Dentin sialophosphoprotein (DSPP)*, expressed by odontoblasts, reveals a number of *Runx2* binding sites (Chen et al, 2002). The function of *Runx2* during development has been elucidated by the generation of $Runx2^{-/-}$ mice, which completely lack bone formation and exhibit arrested tooth development (Otto et al, 1997). Teeth of $Runx2^{-/-}$ mice are malformed and hypoplastic, lack normal odontoblast and ameloblast differentiation without formation of regular enamel and dentin matrices, indicating the function of *Runx2* in the morphogenesis of the differentiating enamel organ (D'Souza et al, 1999).

ABNORMALITIES OF TOOTH SHAPE

Abnormalities of tooth shape include taurodontism, dens invaginatus, microdontia, dens evaginatus, talon cusps and double formation of teeth. Taurodontism (MIM 272700), meaning bull teeth, is an abnormality characterized by a tooth shape with a single root, and conic molars with an increased pulp chamber size (Fig. 8). Taurodontism results from inadequate differentiation of the epithelium, confined typically to the upper and lower permanent molars. It appears in an isolated form and also occurs in association with other conditions.

Trichodentoosseous syndrome (TDO; MIM 190320) is characterized by kinky hair, taurodontism, brittle nails

and mild to moderate increased bone density and is caused by *DLX3* mutations (Price et al, 1998). *Dlx3* is expressed in the mesenchymal tooth forming cells that give rise to dentin and the pulp (Robinson and Mahon, 1994). At the late bell stage, *Dlx3* expression is shifted to the inner enamel epithelium that gives rise to the ameloblasts, responsible for enamel formation, consistent with the thin pitted enamel TDO phenotype. In addition, the inner enamel epithelium forms the Hertwigs root sheath, necessary to establish root morphology. Failure of Hertwigs root sheath to invaginate at the appropriate time leads to taurodontism, present in TDO.

Dens invaginatus is a developmental anomaly characterized by a deep surface invagination of the crown or root that is lined by enamel. The malformation shows a broad spectrum of morphologic variations and frequently results in early pulp necrosis. Conversely, dens evaginatus results from improper outfolding of the enamel organ and is characterized by a tuberculated appearance of the lingual/occlusal surface of affected anterior/posterior teeth. Similarly, in talon cusps an accessory cusp like structure projects from the cingulum to the cemento-enamel function. Talon cusps may occur in both primary and secondary dentition and often lead to occlusal interferences, occlusal trauma and acute periodontitis. They are more common in Asians and are also a feature of Rubinstein-Taybi syndrome (Hennekam and Van Doorne, 1990). A few familial cases of dens evaginatus have been reported; however, so far no molecular findings have been reported for dens in- or evaginatus or isolated talon cusps.

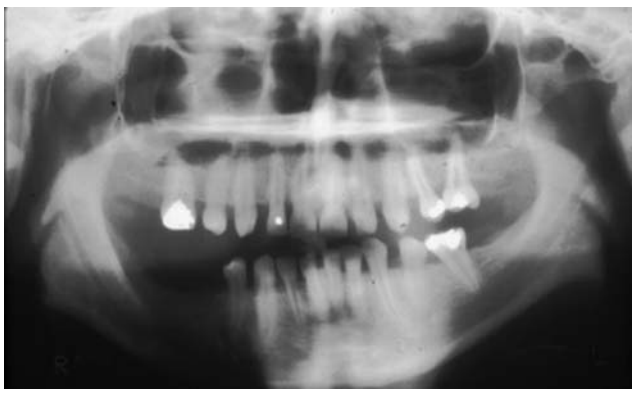

Fig. 8 Hypodontia and Taurodontism. Orthopantomogram showing hypodontia with taurodontism. Taurodontic teeth exhibit only a single root, conic molars and characteristic pulp changes.

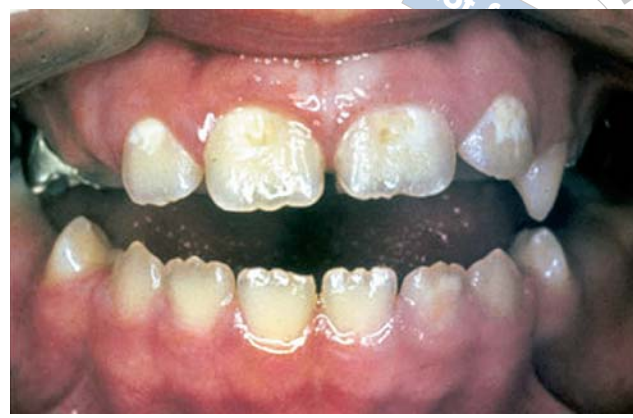

Fig. 9 Amelogenesis imperfecta hereditaria (AIH). Patient with amelogenesis imperfecta with hypoplastic defects, presenting as grooved enamel with hypomineralization.

ABNORMALITIES OF TOOTH STRUCTURE

Abnormalities of tooth structure include mineralization defects of enamel and dentin. Defects in enamel and dentin formation are usually found as separate entities. They may occur in an isolated form as in amelogenesis imperfecta and dentinogenesis imperfecta or in association with distinct developmental syndromes

Amelogenesis imperfecta

Enamel is a highly mineralized tissue and consists of about 98% of large, strictly organized hydroxyapatite crystals. Enamel is produced by the epithelial organ of ameloblasts and is a unique material found in teeth. Amelogenesis imperfecta (AIH) summarizes a number of inherited conditions characterized by abnormal enamel formation that show marked clinical and genetic heterogeneity with various distinct clinical subtypes. The teeth of affected individuals show hypoplastic enamel, which is thin but appears normal, hypomineralized enamel or overlapping forms (Fig. 9). AIH may be inherited in an autosomal recessive, dominant, or X-linked trait, however, no apparent correlation between the different forms and their phenotypes exists.

A deletion of the X-chromosomal amelogenin *(AMELX)* gene has been shown to cause the X-linked recessive AIH1 in one family (MIM 301200) (Lagerstrom et al, 1991). AIH1 is characterized by hypoplastic defects, presenting as thin pitted or grooved enamel and/or hypomineralization where the enamel content is decreased. The importance of *Amelx* in enamel organization was demonstrated by the generation of *Amelx*$^{-/-}$ mice exhibiting a hypoplastic en-

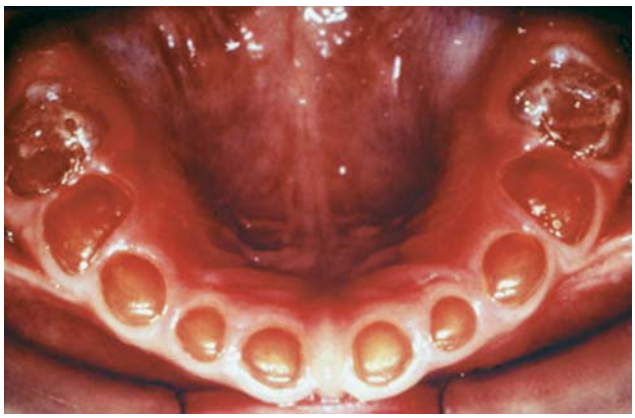

Fig. 10 Dentinogenesis imperfecta (DGI). Patient with dentinogenesis imperfecta type II with characteristic opalescence of dentin and loss of enamel.

amel layer with loss of the characteristic prism pattern (Gibson et al, 2001).

An autosomal dominant local hypoplastic form of AIH has been mapped to human chromosome 4q11-q21, designated AIH2 (MIM 104500) and is caused by mutations in *Enamelin (ENAM)* (Rajpar et al, 2001). Similar to *Amelx, Enam* is expressed throughout early stage maturation of ameloblasts until day 9. However, *Amelx* continues to be expressed in the maturation stage until day 14 (Hu et al, 2001). Both genes are refined exclusively to enamel indicating that they do not participate in the formation of dentin or cementum in developing molars.

In addition, enamel defects may occur in association with a number of syndromes such as: Ectodermal dysplasias, Lacrimo-auriculo-dental-digital syndrome (LADD; MIM 149730); Oculo-dento-digital syndrome (ODDD; MIM 164200); and Oro-facio-digital syndrome I (OFD1; MIM 311200).

Dentinogenesis imperfecta

Dentinogenesis imperfecta (DGI) is characterized by inadequate formation of dentin due to disturbed function and proliferation of odontoblasts in the dental pulp. Odontoblasts represent a single layer of post-mitotic committed cells that secrete an extracellular matrix that is originally unmineralized, referred to as predentin. Predentin is gradually converted to dentin as mineralization of collagen fibrils proceeds by the deposition of hydroxyapatite crystals within and surrounding the fibrils. The composition of dentin extracellular matrix resembles bone in many aspects, including type I collagen, osteonectin, osteocalcin, osteopontin, bone sialoprotein and dentin matrix protein.

Dentinogenesis may appear in an isolated form or in association with osteogenesis imperfecta. The most prevailing isolated form is dentinogenesis imperfecta type II according to the Shields classification (DGI-II; MIM 125490). DGI-II is an autosomal dominant disorder with deciduous and permanent teeth showing a blue grey, amber or opalescent discoloration (Fig. 10). Radiographically the teeth exhibit bulbous crowns, narrow roots, and obliterated pulp chambers and root canals. In DGI-II the enamel is also affected, showing a tendency to split from the dentin when imposed to mechanical stress. Dentin dysplasia-II (DTDP2; MIM 125420) is another disorder characterized by dentin mineralization defects. In DTDP2 the deciduous teeth are opalescent as in DGI-II, but the permanent teeth have a normal colour and show large pulp chamber with pulp stones on a radiograph.

Missense mutations have recently been identified in dentin sialophosphoprotein gene *(DSPP)* for DGI-II (Xiao et al, 2001; Zhang et al, 2001) and DTDP2 (Rajpar et al, 2002). *DSPP* is processed into two proteins: dentin sialoprotein (DSP), and dentin phosphoprotein (DPP) that are essential for the dentin extracellular matrix. *Dspp* is expressed in odontoblasts and transiently in preameloblasts (D'Souza et al, 1997) and has recently also been detected in bone (Qin et al, 2003) and in the inner ear (Xiao et al, 2001), explaining the hearing loss seen in individuals with DGI-II (Xiao et al, 2001). $Dspp^{-/-}$ mice exhibit a severe phenotype with enlarged pulp chambers, increased width of the predentin zone, hypomineralization and pulp exposure, similar to human DGI type III (DGI-III; MIM 125500; Sreenath et al, 2003), which is restricted to the Brandywine isolate in southern Maryland. In teeth of patients with DGI-III mineralization does not occur after the montle dentin has been formed and demonstrates enlarged pulp cavities. Interestingly, individuals with DGI-II and III have been reported in the same family (Heimler et al, 1985). These findings indicate that DGI-II and DGI-III may be allelic variants of the same underlying disease. Further molecular analysis will be needed to prove this hypothesis in the future.

SUMMARY AND CONCLUSION

Teeth develop from reciprocal inductive interactions between the epithelium and the mesenchyme. Tooth differentiation and cusp morphogenesis are influenced by the enamel knot and secondary signalling centres. Dif-

ferentiation of odontoblasts and ameloblasts leads to the deposition of enamel and dentin matrices. The identification of genes underlying human syndromes with tooth malformations, the use of genetically manipulated mouse models and tissue recombination experiments have extensively helped to improve the understanding of the molecular signalling network underlying tooth formation and pathology. Indeed, many of the molecular signal transduction pathways in developing teeth are conserved and can be found during development of other organs. In the future, the use of molecular prerequisites may provide a more detailed classification of tooth malformations and hitherto uncharacterised conditions in molecular terms, and a more precise genotype-phenotype correlation. It is conceivable that the molecular insights into tooth development and disease may not only help to improve the understanding of the pathogenesis of tooth malformations, but may also influence therapeutic approaches.

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