Genetic Basis of Nonsyndromic and Syndromic Tooth Agenesis

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Abstract

Human dentition development is a long and complex process which involves a series of reciprocal and sequential interactions between the embryonic stomodeal epithelium and the underlying neural crest–derived mesenchyme. Despite environment disturbances, tooth development is predominantly genetically controlled. To date, more than 200 genes have been identified in tooth development. These genes implied in various signaling pathways such as the bone morphogenetic protein, fibroblast growth factor, sonic hedgehog homolog, ectodysplasin A, wingless-type MMTV integration site family (Wnt), and transform growth factor pathways. Mutations in any of these strictly balanced signaling cascades may cause arrested odontogenesis and/or other dental defects. This article aims to review current knowledge about the genetic mechanisms responsible for selective nonsyndromic tooth agenesis in humans and to present a detailed summary of syndromes with hypodontia as regular features and their causative genes.

Keywords

► tooth agenesis
► hypodontia
► oligodontia
► genes

Introduction

Tooth agenesis affects approximately 2 to 9% of the population,[1] making it one of the most prevalent craniofacial anomalies in humans. Population studies have revealed that up to 20% of the normal population may lose at least one-third molar, and primary dentition is rarely affected than permanent dentition.[1,2] The most frequently absent teeth are third molars (10–25%), followed by lower second premolars and upper lateral incisors.[3] Agenesis of the first and second molars is very rare. When more than six teeth are missing (excluding third molars), the condition is referred to as severe hypodontia or oligodontia. An extreme case of hypodontia is anodontia, denoting complete loss of all teeth.[2] Tooth agenesis can either occur as an isolated condition (nonsyndromic form) or can be occur as part of a genetic syndrome

(syndromic form), reflecting the genetically and phenotypically heterogeneity of the condition. Nonsyndromic tooth agenesis is classified as a sporadic or familial form, inherited in an autosomal dominant, autosomal recessive, or X-linked mode. It is often associated with some other oral defects, such as tooth size and/or shape defects,[2,4] cleft lip (CL) and/or cleft palate (CLP),[5] enamel hypoplasia,[4] and altered craniofacial growth.[5]

Although tooth agenesis is occasionally caused by environmental factors, such as infections, trauma, chemotherapy, or radiotherapy, the majority of cases are due to genetic factors.[6–10] Molecular studies of odontogenesis, using the mouse tooth as a model system, have shown that tooth development is under strict genetic control (►Table 1). During early tooth development, multiple

Table 1 Genes involved in tooth development of mouse models

Gene	Mouse model	OMIM	Type of molecule	Dental phenotypes	Mechanisms
ACTBA	*ActßA*⁻/⁻	102630	Signal molecule	Arrested at bud stage	Failure of signaling
ACTRIIA	*ActRIIA*⁻/⁻	604221	Receptor	Mandibular incisors agenesis	Failure of signaling
ACTRIIA, ACTRIIB	Double mutant		Receptor	Molar and mandibular incisors agenesis	Failure of signaling
BMPR1A	*Bmpr1a*⁻/⁻	601299	Receptor	Arrested at early bud stage	Impaired BMP signaling pathway
DKK1	Overexpression	605189	Extracellular matrix	Arrested before or at placode stage	Decreased Wnt signaling
DLX1/DLX2	Double ko		Transcription factor	Arrested before or at placode stage	Failure of signaling
EDA	Tabby mutant	300451	Signal molecule	Partial agenesis, abnormal size, shape	Abnormal epithelial and enamel knot signaling
	Overexpression			Supernumerary teeth	Overactivation of EDA signaling
EDAR	*Downless* mutant	604095	Receptor	Partial agenesis, abnormal size, shape	Abnormal epithelial and enamel knot signaling
EDARADD	*Crinkled* mutant	606603	Intracellular protein	Partial agenesis, abnormal size, shape	Abnormal epithelial and enamel knot signaling
EVC	*Evc*⁻/⁻	604831	Intracellular protein	Agenesis of maxillary incisors	Abnormal hedgehog signaling
FGF8	Conditional ko	600483	Signal molecule	Molar agenesis	Failure of signal transmission
FGFR2	*Fgfr2IIIb*⁻/⁻	176943	Receptor	Arrested at bud stage	Failure of signal transmission
FST	*Fst*⁻/⁻	136470	Extracellular protein	Reduced mandibular incisors	Failure of signal transmission
GAS1	*Gas1*⁻/⁻	139185	Extracellular protein	Fused maxillary incisors	Abnormal sonic hedgehog signaling
GLI2	*Gli2*⁻/⁻	165230	Transcription factor	Fused maxillary incisors	Abnormal sonic hedgehog signaling
GLI2/GLI3	Double mutant		Transcription factor	Arrested before or at placode stage	Abnormal sonic hedgehog signaling
IKKA	*Ikka*⁻/⁻	600664	Intracellular protein	Flat cusp, incisors evaginated	Abnormal epithelial and enamel knot signaling
IKKR	*Ikkr*⁻/⁻	300248	Intracellular protein	Male lethal, female agenesis, or conical teeth	Abnormal epithelial and enamel knot signaling
IRF6	*Irf6*⁺/⁻	607199	Transcription factor	Hypodontia, cleft palate	Affected Jag2-Notch1 signaling
LEF1	*Lef1*⁻/⁻	153245	Transcription factor	Arrested at late bud stage	Abnormal epithelial and enamel knot signaling
MSX1	*Msx1*⁻/⁻	142983	Transcription factor	Arrested at late bud stage	Abnormal BMP signaling
MSX1/MSX2	Double mutant		Transcription factor	Arrested before or at placode stage	Abnormal BMP signaling
P63	*p63*⁻/⁻	603273	Transcription factor	Arrested before or at placode stage	Abnormal epithelial signaling
PAX9	*Pax9*⁻/⁻	167416	Transcription factor	Arrested at bud stage	Failure of signaling
PITX2	*Pitx2*⁻/⁻	601542	Transcription factor	Arrested before or at placode stage	Failure of signaling
PRX1/PRX2	Double mutant	167420	Transcription factor	Arrested before or at placode stage	Abnormal mandibular patterning
RUNX2	*Runx2*⁻/⁻	600211	Transcription factor	Arrested at bud stage	Failure of signaling
SHH	*Shh*⁻/⁻	600725	Signal molecule	Delayed and fused teeth	Failure of signaling
SMAD2	*Smad2*⁺/⁻	601366	Signal transducer	Incisor, mandibular molar agenesis	Failure of signal transduction
SMO	*Smo*⁻/⁻	601500	Signal transducer	Molars fused and delayed eruption	Failure of Shh signal transduction

Abbreviations: BMP, bone morphogenetic protein; EDA, ectodysplasin A; ko, knockout.

signaling molecules are expressed in the dental lamina epithelium and induced the dental mesenchyme.[7,8] Important signaling pathways are involved in organogenesis including, but are not limited to the transcription factors, signaling molecules and its receptors, extracellular matrix molecules, and growth factors.[9] Abnormal gene function (loss-of-function, gain-of-function) may disrupt specific signaling networks and cause a wide variety of selective tooth agenesis patterns or other oral malformations.[10] Some genes responsible for syndromic tooth agenesis, including ectodermal dysplasias (EDs), Rieger and Witkop syndrome (►**Table 2**), as well as those causing inherited oligodontia as an independent trait, have been identified.

Genes Involved in Nonsyndromic Tooth Agenesis

Muscle Segment Homeobox, Homolog 1 (*MSX1*)

Muscle segment homeobox, homolog 1 (*MSX1*) belongs to a family of transcription factor that contains a highly conserved DNA-binding homeodomain.[11,12] The principle phenotypes in homozygous *MSX1*-deficient mice were complete secondary CLP, failure of incisor development, and bud-stage arrest of molar development,[13] suggesting that *MSX1* is involved in multiple epithelial–mesenchymal interactions during mouse embryogenesis and appears to be critical during early tooth germ (bud and cap) developmental stages.[12,13]

Table 2 Human syndromic tooth agenesis and known genes

Gene	Type of molecule	Syndrome	OMIM	Dental/oral phenotypes	Mechanisms
ADAMTS2	Procollagen I N-proteinase	Ehlers–Danlos hypermobility type VII	225410	Hypodontia, microdontia, dentin dysplasia, severe gingival hyperplasia, open bite, TMJ mobility restriction	Unknown
ANTXR1	Tumor-specific endothelial marker	GAPO syndrome	230740	Pseusoanodontia, growth retardation, alopecia	Defect in extracellular matrix homeostasis
BCOR	Transcription regulator	Oculo-facio-cardio-dental syndrome	113650	Male lethal, no tooth development, female fused teeth	Abnormal transcription regulator
COL1A1/2	Extracellular matrix	Osteogenesis imperfect type I	166200	Hypodontia	Abnormal extracellular matrix
CREBBP	Transcriptional coactivator	Rubinstein–Taybi syndrome	180849	Hypodontia, hyperdontia, natal teeth, micrognathia	Inactivate transcription of cAMP responsive genes
COL3A1	Collagen	Ehlers–Danlos hypermobility type	130020	Hypodontia of permanent mandibular incisors, dentin dysplasia	Abnormal collagen I fibrillogenesis
CXORF5	Intracellular protein	Orofacial digital syndrome type I	311200	Male lethal, female hypodontia	Cilia formation failure
DTDST	Sulfate transporter	Diastrophic dysplasia		Hypodontia	Impaired proteoglycan synthesis
EDA	Signal molecule	Hypohidrotic ectodermal dysplasia	305100	Oligodontia/anodontia, peg or misshaped teeth	Abnormal epithelial and enamel knot signaling
EDAR	Receptor	Hypohidrotic ectodermal dysplasia	129490	Oligodontia/anodontia, peg or misshaped teeth	Abnormal epithelial and enamel knot signaling
EDARADD	Intracellular protein	Hypohidrotic ectodermal dysplasia	224900	Oligodontia/anodontia, peg or misshaped teeth	Abnormal epithelial and enamel knot signaling
EVC	Intracellular protein	Ellis–van Creveld syndrome	225500	Agenesis of incisors, conical teeth, taurodontism	Abnormal hedgehog signaling
EVC2	Intracellular protein	Ellis–van Creveld syndrome; Weyers syndrome	225500	Agenesis of incisors, conical teeth, taurodontism	Abnormal hedgehog signaling
EYA1	Phosphatase	Branchio-oro-renal syndrome	113650	Branchial fistulas or cysts, hearing loss	Disrupt phosphorylation-dependent transcription factor modulation
FGF10	FGFs	Lacrimoauriculodentodigital syndrome	149730	Enamel dysplasia, microdontia, hypodontia	Failure of FGF signaling
FGFR2	FGFs receptor	Apert syndrome	101200	Hypodontia	Abnormal epithelial and enamel knot signaling
FGFR3	FGFs receptor	Crouzonodermoskeletal syndrome	612247	Hypodontia, delayed eruption, cementomas	Unknown
FLNB	Filamin B	Larsen syndrome	150250	Delayed eruption, flattened midface, cleft palate	Affect segmentation and endochondral ossification

Table 2 (*Continued*)

Gene	Type of molecule	Syndrome	OMIM	Dental/oral phenotypes	Mechanisms
FOXC1	Transcription factors	Axenfeld–Rieger syndrome type 3	602482	Oligodontia, microdontia, and short roots	Impaired Notch signaling
GJA1	Gap junction protein	Oculodentodigital dysplasia	164200	Small teeth	Abnormal gap junctions
GRHL2	Transcription factor	Autosomal recessive ectodermal dysplasia	616029	Hypodontia, enamel hypoplasia	Unknown
HOXB1	Transcription factor	Moebius syndrome	157900	Facial paresis, missing teeth, ectrodactyly	Disrupted DN-protein binding, altered transcriptional activity
IKBKG	Intracellular protein	Hypohidrotic ectodermal dysplasia with immune deficiency	300291	Agenesis or conical teeth	Abnormal epithelial and enamel knot signaling
IKKγ	Intracellular protein	Incontinentia pigmenti	308300	Agenesis or conical teeth in females	Abnormal epithelial and enamel knot signaling
IRF6	Transcription factor	Van der Woude syndrome	211370	Hypodontia	Failure of signaling
JAG1	Notch receptor ligand	Alagille syndrome	118450	Oligodontia, coronal craniosynostosis	Impaired dominant-negative inhibition of Notch signaling
KMT2D	Lysine (K)-specific methyltransferase	Kabuki syndrome	147920	Missing incisors, cleft lip/palate, bifid tongue, and uvula	Disrupted histone methylation
KDM6A	Lysine (K)-specific demethylase		300867		Impaired histone demethylation
MKKS	Chaperonin	Laurence–Moon–Bardet–Biedl syndrome	245800	Hypodontia, small teeth, short roots	Unknown
NSD1	Androgen receptor	Sotos syndrome	117550	Premolar missing, enamel hypoplasia	Unknown
P63	Transcription factor	P63 syndromes	129900	Hypodontia	Failure of epithelial signaling
PHGDH	Phosphoglycerate dehydrogenase	Neu–Laxova syndrome	256520	Hypodontia	Unknown
PITX2	Transcription factor	Rieger syndrome type I	180500	Oligodontia	Failure of epithelial signaling
PORCN	Transmembrane protein	Focal dermal hypoplasia	305600	Lip papillomas, hypoplastic teeth	Abnormal Wnt signaling
PVRL1	Adhesion protein	Cleft palate–ectodermal dysplasia syndrome	225060	Oligodontia	Perturbed cell adhesion
RECQL4	DNA helicase	Rothmund–Thomson syndrome	268400	Hypodontia	Unknown
RSK2	Ribosomal kinase	Coffin–Lowry syndrome	303600	Thick lips, high palate, hypodontia, microdontia, delayed eruption, premature tooth loss	Abnormal phosphorylation and activation of MAPK signaling
SHH	Signal molecule	Holoprosencephaly	142945	Fused teeth and delayed eruption	Failure of signaling
TBX3	Transcription factor	Ulnar mammary syndrome	181450	Hypodontia	Failure of epithelial signaling

(*Continued*)

Table 2 (Continued)

Gene	Type of molecule	Syndrome	OMIM	Dental/oral phenotypes	Mechanisms
TFAP2B	Transcription factor	Char syndrome	169100	Agenesis of premolar or molars	Reduced transcription
TCOF1	Intracellular protein	Treacher–Collins syndrome	154500	Hypodontia	Abnormal nuclear trafficking
WNT10A	Signal molecule	Odonto-onycho-dermal dysplasia	257980	Oligodontia, abnormal shape and size	Failure of signaling
UBR1	Recognin	Johanson–Blizzard syndrome	243800	Oligodontia, nasal alae aplasia, hearing loss	Unknown

Abbreviations: cAMP, cyclic adenosine monophosphate; FGF, fibroblast growth factor; GAPO, growth retardation, alopecia, pseudo-anodontia, optic atrophy; TMJ, temporomandibular joint.

MSX1 was the first causative gene identified in human nonsyndromic tooth agenesis.[14] To date, more than 28 *MSX1* heterozygous mutations have been reported to cause various types of selective tooth agenesis, suggesting that the mutant phenotype was due to haploinsufficiency, rather than a dominant-negative mechanism. Among these mutations, the majority are missense or nonsense mutations. The phenotype caused by a premature stop codon was slightly more severe than that caused by the substitution of one amino acid. This might be attributed to the increased susceptibility of degradation and lack of stability of the truncated protein messenger RNA (mRNA) (nonsense-mediated decay) compared with the missense mutated protein. The most distinguishing feature of *MSX1*-associated tooth agenesis is the frequent absence of second premolars and third molars (75%). Only one case of autosomal recessive oligodontia is known for a *MSX1* missense mutation.[15] In addition to isolated tooth agenesis, mutations of *MSX1* cause other phenotypes. Some affected individuals also had CL or CLP, extending the *MSX1* mutations phenotypes and supporting relationship between *MSX1* and nonsyndromic CL and CLP.[14] A *MSX1* Ser202-Stop mutation was reported to be associated with the Witkop syndrome, which includes tooth agenesis and nail dystrophy.[12] Most *MSX1* mutations (70%) causing isolated tooth agenesis clustered in the homeodomain, whereas all mutations associated with CL and/or CLP clustered outside the homeodomain. Also, abnormalities of the short arm of chromosome 4 including deletion of the entire *MSX1* gene can cause Wolf–Hirschhorn syndrome, which exhibited oligodontia as part of the phenotype.[16] These indicate that mutations in different domains of *MSX1* have different effects during craniofacial development. The exact mechanisms through which variant forms of *MSX1* mutations lead to variable phenotypes remain unknown. It is speculated that interactions of mutant *MSX1* with other regulatory proteins such as *DLX2* and *DLX5*, *LHX2*, *PAX3*, and *PAX9* may have been disturbed.

Paired Box Gene 9 (*PAX9*)

PAX9 is a transcription factor characterized by a paired domain, an octapeptide motif, and a C-terminal transcriptional regulatory domain. *PAX* genes all share a unique paired domain which is an evolutionarily conserved 128-amino-acid region binding with DNA in a sequence-specific manner. This paired domain consists of two independent subdomains that structurally resemble a helix-turn-helix motif.[17] *PAX9* is a key regulator in mouse embryogenesis. It is widely expressed in the neural crest–derived mesenchyme that involved in craniofacial and tooth development, and is required for expression of BMP4, *MSX1*, and *LEF1*.[18] *PAX9* null mice die shortly after birth. In addition, all teeth fail to develop beyond the bud stage. Secondary CLP and other skeletal abnormalities in the head and limbs also have been observed.[18] Another *PAX9*-neo mouse model which produced decreased levels of *PAX9* mRNA and showed oligodontia. Homozygous *PAX9* neo/neo mice exhibited hypoplastic or missing lower incisors and third molars. When combined with a *PAX9* null allele, the *PAX9* neo/null mutant mice developed severe oligodontia.[19] The missing molars were arrested consistently at an earlier stage, suggesting that a reduction of *PAX9* dosage may be essential in tooth development. *PAX9* neo/neo and neo/null

mice also showed defects in enamel formation of the continuously growing incisors, whereas molars exhibited increased attrition and reparative dentin formation.[20]

So far, more than 20 *PAX9* mutations have been detected, most of them located in the paired domain. These mutations, ranging from missense to premature terminations or even deletion of the whole gene, are all heterozygous, indicating that a loss-of-function mechanism contributed to haploinsufficiency of *PAX9*.[21] Unlike the phenotypes caused by *MSX1* mutations, *PAX9* mutations preferentially lead to second molars absence,[20,21] and sometimes in combination with other types of tooth agenesis, most commonly second premolars and mandibular incisors.[3,10] Moreover, it has been reported that missense mutations in the paired domain clustered near the N-terminal resulted in the molar and canine agenesis phenotype. The precise mechanism about *PAX9* mutations is still unknown, mutant *PAX9* may impair DNA binding and by modifying downstream bone morphogenetic protein (BMP)4 promoter transcriptional activities.[20]

MSX1 and PAX9 Interactions

Multiple lines of evidence have demonstrated that *MSX1* and *PAX9* are dosage-sensitive genes and can interact both at the gene and protein levels.[22] *PAX9* interacts synergistically with *MSX1* to activate both *MSX1* and the downstream BMP4 promoter in vitro.[23] BMP4 is a key *MSX1*-dependent mesenchymal odontogenic signal for driving tooth morphogenesis through the bud-to-cap transition and enamel knot induction at the late cap stage.[24] Mutations in either *PAX9* or *MSX1* can lead to defective interactions that disrupt normal BMP4 expression and function which is essential for the tooth morphogenesis. A recent in vivo study showed that *PAX9* (+/−) and *MSX1*(+/−) double mutants exhibited consistently absent lower incisors and an incompletely penetrant CL phenotype and transgenic BMP4 expression can partly rescue this phenotype.[24,25] These data suggest that *MSX1* and *PAX9* function together throughout tooth and craniofacial development and affect multiple signaling pathways that influence incisor size and symmetry. Also, a combined reduction of *PAX9* and *MSX1* gene dosages may increase the risk for orofacial clefting and oligodontia.[25] Recently, it was revealed that a zinc finger transcription factor odd-skipped-related 2 acts downstream of *PAX9* and patterns the mesenchymal odontogenic field through protein–protein interactions with *MSX1* and *PAX9* during early tooth development.[26]

Axis Inhibitor 2 (AXIN2)

Axis inhibitor 2 (*AXIN2*) is an important negative regulator of catenin Wnt signaling pathway. Mutations in genes of the Wnt signaling pathway that prevent the degradation of β-catenin can lead to tumorigenesis. Somatic *AXIN2* mutations have been described in a variety of human cancers, including skin, gastrointestinal, liver, and ovarian cancer.[27] In mouse embryos, Axin2 is expressed intensively in dental papilla mesenchyme and enamel knots during odontogenesis, suggesting the involvement of Axin2 in regulating tooth formation.[27,28] Reports of *AXIN2* mutations in isolated tooth agenesis are rare in comparison with investigations of other causative genes. The first germline *AXIN2* mutation was identified in a large Finnish family presenting isolated oligodontia and a variable colorectal phenotype with full penetrance. In this family, the oligodontia phenotype was entirely penetrant in mutation carriers. No ectodermal findings were described. Almost all family members with oligodontia had colorectal neoplasms, ranging from polyposis to colorectal malignancy with no polyps.[27,29] Another de novo germline mutation was also detected in a 13-year-old boy with oligodontia. Of note, this mutation was identical to a frameshift mutation described in a colorectal cancer tissue. In another three-generation family segregating autosomal dominant oligodontia variably associated with sparse hair and eyebrows, colon or gastric polyps, early-onset colorectal, and/or breast cancer, a novel heterozygous nonsense mutation was identified in *AXIN2*.[30] Subsequently, *AXIN2* mutations were reported in patients with syndromic or nonsyndromic tooth agenesis and oral clefts.[3,27] A significant association between *AXIN2* variants and cases with at least one missing incisor were also identified.[3] When compared with the pattern of agenesis of the posterior dentition seen in *MSX1* and *PAX9* affected families, individuals with loss-of-function mutation in *AXIN2* display a mixed pattern of dental agenesis (►**Figs. 1** and **2**).[31]

Ectodysplasin A (EDA)

X-linked hypohidrotic ED (HED) (XLHED), the most frequent form of HED, is characterized by abnormalities of teeth, hair, and eccrine sweat glands.[32] Affected males usually present all of the typical phenotypes, including frontal bossing, sparse hair, and dry skin. Teeth are often missing or misshapen. In heterozygous female carriers, however, the severity of the disorder varies widely, even within families. The gene responsible for XLHED was ectodysplasin A (*EDA*). EDA is a type II transmembrane protein with a small N-terminal intracellular domain, a larger extracellular domain containing a (Gly-X-Y)$_{19}$ collagen-like repeat, and a C-terminal tumor necrosis factor (TNF) homology domain.[32] EDA, being a member of the TNF ligand superfamily, has extensive alternative splicing that leads to various isoforms. The most common EDA splice isoforms (EDA-A1 and EDA-A2) promote transcription by binding to distinct receptors.[32] That is, EDA-A1 binds to the EDA receptor (EDAR), which interacts with the EDAR-associated death domain (EDARADD) and activates the downstream nuclear factor-κB (NF-κB) kinase signaling pathways.

More than 100 mutations have been reported in *EDA*, most of which are localized within exons 1, 3, and 5 and are mainly point mutations, clustering in the four functional domains of EDA. Intragenic insertions or deletions, large deletions including entire exon loss, and complete gene deletion have also been reported. Recently, mutations of EDA have been reported in some X-linked nonsyndromic hypodontia families.[33] Unlike patients previously described with XLHED, affected patients from both families did not have any clinical features except oligodontia, and almost all female carriers were normal or had a milder phenotype, suggesting that *EDA* mutations can lead to a unique form of isolated X-linked recessive hypodontia.[34,35]
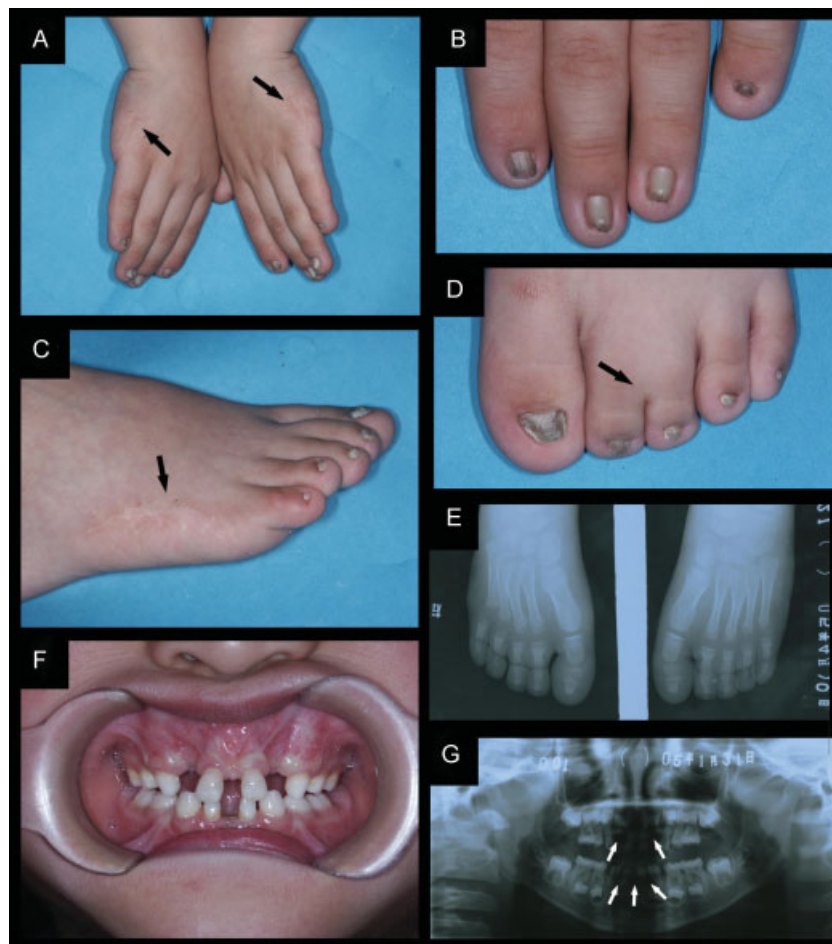
Fig. 1 Clinical photographs and radiographs of a patient affecting with Weyers acrofacial dysostosis. (A and C) Bilateral postaxial polydactyly type A was removed at birth by surgical operations on both hands and feet, only scars (black arrows) were seen after amputation. (B and D) Apparent dysplastic fingernails and toenails. (D and E) Partial syndactyly at the second and third toes on both feet (black arrows). X-ray shows that the phalanges were not affected. (F) Oral examination of the upper lip reveals multiple oral frenula, diastemas, teeth which were abnormal in size and shape, delayed teeth eruption. (G) Panoramic radiographs showed the absence of permanent incisors (white arrows).

Wingless-Type MMTV Integration Site Family, Member 10A (*WNT10A*)

Odonto-onycho-dermal dysplasia (OODD) is a rare autosomal recessive syndrome mainly characterized by dry hair, oligodontia, smooth tongue, onychodysplasia, keratoderma, and hyperhidrosis. Schöpf–Schulz–Passarge syndrome (SPSS) is distinguished by the presence of multiple eyelid cysts, histologically corresponding to apocrine hidrocystomas. A homozygous nonsense mutation in *WNT10A* was found in all patients from three consanguineous families affecting OODD[36–38] and an allelic disease SPSS. This demonstrated that ED can be caused by *WNT10A* which altered the Wnt signaling pathway. *WNT10A* is strongly expressed in embryonic limb, skin, hair follicle, and dental epithelium at the dental lamina and bud stages.[39] During cap stage, its expression in the enamel knot is marked.

Recently, a large proportion ($> 50\%$) of patients with isolated oligodontia and mild manifestations of ED were reported with biallelic or monoallelic *WNT10A* mutations,

In an American family with variable hypodontia involving the lateral incisors and premolar teeth missing, two missense mutations F228I and D217N in *WNT10A* were identified, for which affected members were heterozygous or compound heterozygous. None of the family members had other ED manifestations.[40] Recently, *WNT10A* mutations were found in patients with nonsyndromic tooth agenesis.[41] The most frequent mutation, F228I, represented 62% of the *WNT10A* mutations in these nonsyndromic hypodontia patients. Individuals with biallelic mutations had a relatively severe phenotype than those with monoallelic mutations. Upper and lower premolars were the most affected missing teeth. Probands with biallelic mutations had a higher frequency of absent maxillary and mandibular molars and mandibular central incisors. In six Thai patients with isolated hypodontia of the maxillary permanent canines, three heterozygous mutations were identified in *WNT10A*. One of the affected individuals also had pegged maxillary permanent lateral incisors with dens invaginatus. Two mothers of the patients
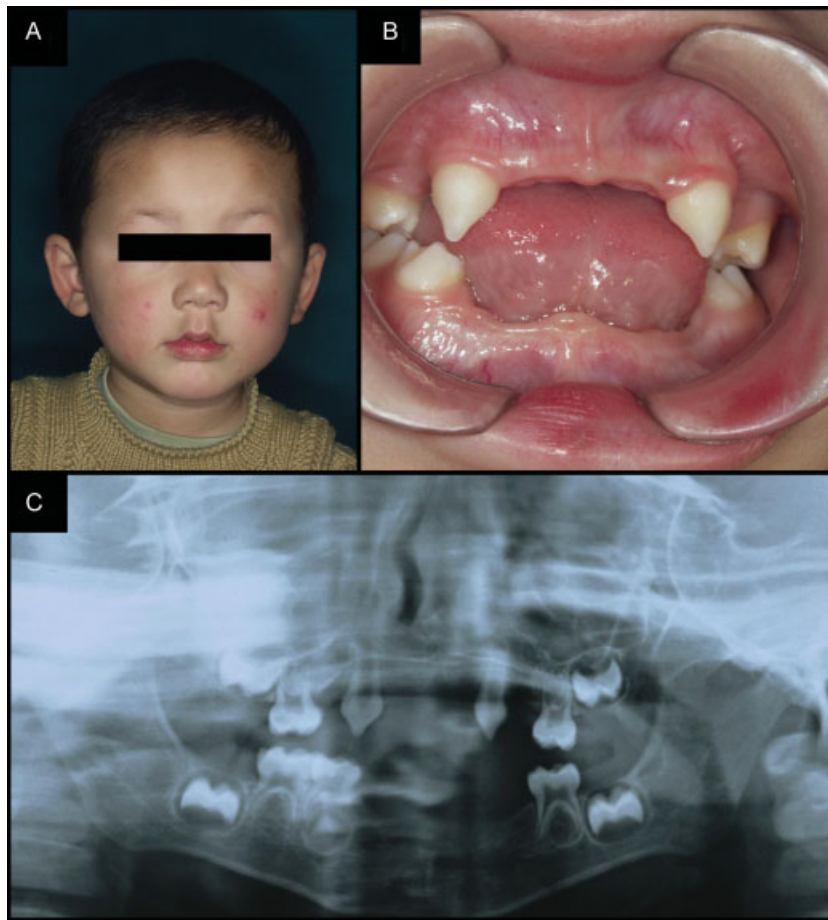
Fig. 2 Characterization of a patient with X-linked recessive isolated hypodontia. (A and B) The patient is a 13-year-old boy, who had fine scalp and body hair, nails, and no complaints about tolerance to heat. His facial features appeared normal, but dental examination revealed multiple missing and misshapen deciduous teeth. (C) A panoramic radiograph depicts the typical pattern of congenitally missing teeth and tooth buds.

carried the mutation and had pegged maxillary permanent lateral incisors,[41] indicating that *WNT10A* is a major gene in the etiology of isolated hypodontia.

Latent Transforming Growth Factor β-Binding Protein 3 (*LTBP3*)

Latent transforming growth factor β-binding protein 3 (*LTBP3*) was first identified through its similarity to human fibrillin-1 epidermal growth factor-like repeats. It is an extracellular matrix protein and binds to the latent form of the multipotent cytokine transform growth factor (TGF)-β. Several genes in the TGF-β signaling pathway have been implicated in skeletal developmental disorders, emphasizing the important role of this pathway in bone growth. Through genome-wide linkage study and sequencing, a homozygous nonsense mutation was identified in *LTBP3* causing an autosomal recessive form of familial severe oligodontia, short stature, as well as apparent increased bone density in the spine and skull base.[42,43] These findings suggested key roles for *LTBP3*-mediated transcription in development of the human tooth and axial skeleton. However, *LTBP3* null mice had no obvious dental abnormalities comparable to the human phenotypes. The adult *LTBP3* null mice were more than 50% smaller than sex-matched wild-type littermates. By

day 12, null mice developed craniofacial malformations. At 3 months, there was a pronounced rounding cranial vault, shortened upper jaw, underbite, and kyphosis. The mutant mice also developed osteosclerosis of the long bones, thoracic kyphosis, distorted ribcage along with cranial base synchondroses. Between 6 and 9 months of age, mutant mice also developed osteosclerosis and osteoarthritis. These subsequent phenotypic changes were consistent with those described for mice that have perturbed TGF-β signaling.[44] The mechanism responsible for the synostosis in *LTBP3* null mice is not clear. *LTBP3* may have a structural function within the synchondroses, which in the absence of *LTBP3* ossify prematurely, or *LTBP3* may play an important role in regulating chondrocyte differentiation by controlling the TGF-β availability in the synchondrosis, and decreased TGF-β level may regulate parathyroid hormone-related protein expression either downstream of Indian hedgehog (Ihh) or independently of Ihh signaling.[45]

SPARC-Related Modular Calcium-Binding Protein 2 (*SMOC2*)

Secreted protein acidic and rich in cysteine (SPARC)-related modular calcium-binding protein 2 (*SMOC2*) is an early dental developmental gene in human beings. It belongs to a family of

BM-40 matricellular proteins which is known as SPARC, and regulates interactions between cells and the extracellular matrix. *SMOC2* expression was found in the oral ectoderm and the outer dental epithelium at E14.5 in mouse and in mesenchymal papilla facing the epithelial loops of molars and the only lingual loop of incisors.[46] Recently, by genome-wide scan and Sanger sequencing, a homozygous splicing mutation was detected in a highly consanguineous Turkish family which manifested extreme microdontia, oligodontia, and dental dysplasia type I.[46] The unaffected parents and siblings were heterozygous for the mutation. A second nonsense *SMOC2* mutation was reported in a consanguineous Pakistan family with autosomal recessive oligodontia and microdontia.[47] Although the function of *SMOC2* in mammalian development has yet to be fully investigated, there are some evidences that it may affect the receptor-mediated signaling of many growth factors. Knockdown of *SMOC2* in zebrafish showed that the first two bilateral teeth were smaller than those of the controls at day 5, the size and presence of the teeth were probably dependent on the level of *SMOC2* depletion, and affected pharyngeal teeth development which had abnormalities resemble of the human phenotype. Expression of three major odontogenesis genes altered: *DLX2*, *BMP2*, and *PITX2*,[47] suggesting that *SMOC2* has an evolutionarily conserved role in tooth and oropharyngeal development.

Other Genes

EDAR and *EDARADD* are two genes that can cause autosomal dominant as well as autosomal recessive HED and nonsyndromic tooth agenesis.[48–50] Keratin 17 (*KRT17*) is expressed in the sebaceous gland, nail bed, hair shaft, and epidermal appendages. Mutations in *KRT17* have been identified in patients with pachyonychia congenita type 2 and oligodontia.[51] *FGFR1*, transmembrane receptor of fibroblast growth factors (FGFs), one of the genes that is also involved in human craniosynostosis, has been associated with families of nonsyndromic hypodontia, in particular, premolar agenesis.[52] *FGFR1* is broadly expressed in the facial primordia and plays important roles in advancing skeletogenesis by regulating osteoblast and chondroblast differentiation.[52]

Syndromic Tooth Agenesis and Genes

More than 200 CL/CLP syndromes exhibit varying levels of hypodontia as part of their phenotypes. The prevalence of hypodontia increases with cleft severity, and upper lateral incisor is the most frequently affected tooth in the cleft area both in primary and in permanent dentitions. A higher incidence of dental agenesis outside the cleft region, more in particular involving the permanent maxillary dentition has also been reported. At present, several syndromic CLP-specific gene mutations have been identified (►**Table 2**); however, the role of these genes in the development of dental agenesis remains unclear.

Van der Woude Syndrome

Van der Woude syndrome (VWS) is one of the most common human autosomal dominant disorders associated with CL/

CLP (∼1% of syndromic CL/CLP cases), characterized by lower lip pits, CL/CLP, and hypodontia (in 70% of cases). Mutations in interferon regulatory factor 6 (*IRF6*) have been identified in more than 100 unrelated families with VWS. *IRF6* encodes a transcription factor that is highly expressed in a variety of embryonic craniofacial tissues, including the medial edges of the fusing palatal processes, tooth buds, hair follicles, and skin, and it has been suggested that *IRF6* mediates interactions between members of the TGF-β superfamily of signaling peptides.[53]

P63 Syndromes

P63 gene mutations have been found in several oral clefting syndromes, which are namely *P63* syndromes. Ectrodactyly–ED–clefting (EEC) syndrome is a rare autosomal dominantly inherited condition characterized by split hands and feet, ED, and CLP. Additional EEC features include lacrimal duct anomalies, urogenital defects, conductive hearing loss, chronic respiratory infections, ventricular cardiomyopathy, and developmental delay. Ankyloblepharon ectodermal defects CL/CLP syndrome, limb mammary syndrome, acrodermato-ungual-lacrimal-tooth syndrome, and Rapp–Hodgkin syndrome are EEC-like conditions and allelic to the EEC syndrome. Several different mutations of *P63* gene have been revealed in these families.[54]

CLP–Ectodermal Dysplasia Syndrome

A homozygous loss-of-function mutation in the *PVRL1* gene has been linked to CLPED-1, an autosomal recessive CLP–ED syndrome. Patients with CLPED-1 have scanty eyebrows, sparse and dry hair, syndactyly of the fingers and toes, CL/CLP, nail dysplasia, hypodontia of the upper central incisors, and abnormal size and shape of tooth crowns.[55]

Ectodermal Dysplasia Syndromes

The term ED is used to designate a heterogenous group of disorders characterized by a constellation of findings involving a primary defect of the skin, teeth, and appendageal structures including hair, nail, exocrine, and sebaceous glands. The different types of ED are caused by the mutation or deletion of certain genes located on different chromosomes.

HED is the most common ED, usually inherited as an X-linked recessive trait, or inherited in autosomal dominant or recessive forms. X-linked recessive HED is caused by mutations in *EDA* gene. The protein product, EDA, is a novel member of the TNF family and functions as a signaling molecule during epithelial morphogenesis.[48] Autosomal dominant and recessive forms of HED are caused by mutations in *EDAR* or *EDARADD* coding for a TNF receptor.

Incontinentia pigmenti (IP) is a rare multisystem disorder classified as an ED condition with additional ocular and central nervous anomalies. IP is an X-linked dominant disorder due to mutations in *NEMO* gene. Mostly, females are affected as males with the mutation usually do not survive through gestation. More than 90% of patients with IP have associations of oligodontia, microdontia, and supernumerary cusps in the posterior permanent teeth, delayed tooth eruption, and/or taurodontia. A distinct form of HED with immunodeficiency

segregates as an X-linked recessive trait, which is allelic to IP. NEMO, the protein in focus, is required for activation of NF-κB, a transcription factor transducing TNF signaling.

Witkop syndrome is another autosomal dominant ED characterized by nail dysplasia, severe hypodontia, and conical teeth. Mutations in *MSX1* gene have been identified in several unrelated families with Witkop syndrome.[10]

Other Syndromes

Tooth agenesis not only appears in some common syndromes but also in several syndromes with rather low prevalence, such as Rieger syndrome,[56] Ellis–van Creveld syndrome,[57] and Sotos syndrome (►**Table 2**).

Conclusion

In mammals, tooth development is governed by a sequential and reciprocal signaling process between two adjacent tissues: the primitive epithelium lining the stomodeum and mesenchymal cells arising from cranial neural crest cells.[58] Therefore, genes implicated in epithelial–mesenchymal interactions during different stages of odontogenesis serve as good candidates for tooth agenesis. A variety of dental anomalies, either morphologically, numerically, or structurally may be due to abnormal function of these specific proteins. Recent years, several gene mutations have been detected to be responsible for the tooth agenesis; however, known mutations explain only a restricted number of cases of agenesis, little is known about the genetic defects underlying this complex condition. Oral clefts and syndromic forms of tooth agenesis may be the best models for isolated tooth agenesis. New techniques such as next-generation sequencing are already available for the early diagnosis of mutations that imply a risk of developing genetic diseases. These would bring new possibilities of early diagnosis and treatment.

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