CHAPTER II
REVIEW OF THE LITERATURE

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2.1 Cleft lip with or without cleft palate

2.1.1 Incidence of cleft lip with or without cleft palate

Orofacial clefts are common congenital anomalies. The etiology of orofacial clefts is complex and involves both genetic and environmental factors. Orofacial clefts are consequences of complex mechanisms during early embryological facial development. The incidence of these defects varies according to geographic location, gender, cleft type, ethnicity, and socio-economic status. The incidences in oriental populations are higher than those in Caucasian and Black populations. The incidences in Blacks, Caucasians, Chinese, and Japanese populations are 0.6 (Rahoma, 2007), 1.44 (Derijcke et al., 1996), 2.01 (Wong and King, 1997), and 2.03 (Natsumea et al., 2000) per 1,000 births, respectively. The incidences in Australian, Singaporean and Phillipine populations has been reported to be 1.28 (Chi, 1970), 1.74 (Tan, 1988) and 1.94 (Murray and Bewton, 1977) per 1,000 births, respectively. In the Thai population, the reported incidence ranged from 1.62 (Chuangsuwanich et al., 1998) to 2.49 (Ruangsitt et al., 1993) per 1,000 births.

Orofacial clefts can be unilateral or bilateral, but the left side is more commonly affected than the right. Cleft lip and palate occurs more frequently in males, but cleft palate alone is more common in females (Fogh-Andersen, 1961; Mcleod et al., 2004; Mossey, 2007). It has been reported that approximately 70% of cleft lip and palate cases are non-syndromic. The remaining 30% are syndromic cases, which are associated with one or more additional anomalies (Schutte and Murray, 1999; Gorlin
2.1.2 Classification of cleft lip with or without cleft palate

The most commonly used orofacial cleft classification among surgeons is striped-Y classification (Friedman et al., 1991). This classification is based on the Kernahan and Stark classification (1958). It is a symbolic classification which indicates severity of anatomical and functional deformities (Figure 2.1).

![Figure 2.1 Scheme of symbolic representation for cleft lip and palate (adapted from Friedman, 1991).](image)

2.1.3 Embryology of orofacial development

At the end of the 4th week of gestation, the embryo is 3.5 mm long, while at the end of the 8th week, the embryo is 30.0 mm long and has a fully formed face. The foregut of the embryo is lined by endoderm, and is separated from the stomodeum or oral opening by the buccopharyngeal membrane. This membrane is broken down by the apoptotic process during the 4th week. This breakdown allows the foregut to connect with the oral opening. During the 4th week, a frontal prominence is present in
the midline. It forms the upper boundary of the stomodeum. The olfactory placodes which form the organs of smell on the roof of the nose are located on each side of the frontonasal prominence (Figure 2.2).

![Figure 2.2](image)

**Figure 2.2** Frontal view of facial development during the 4th week. The breaking down of the buccopharyngeal membrane allows the foregut to connect with the oral opening (adapted from Carlson, 2004).

Two nasal placodes are elevated into a horseshoe shape during the 5th and 6th weeks, and become the medial and lateral nasal processes. The medial nasal processes grow more rapidly than the lateral ones, and fuse with each other in the midline. The fused medial processes becomes the central part of upper lip, alveolar process, maxillary incisors, and anterior palate in front of the incisive foramen (primary palate). The lateral nasal processes develop into the alae of the nose. Invaginations of the center of the medial and lateral nasal processes become the nasal pits, and subsequently develop into the nostrils (Figure 2.3). The inner aspect of the maxillary process on each side develops into a palatal shelf (Figure 2.4). Two mandibular processes swell and project to the midline, and fuse with each other to form the mandibular arch and the lower lip (Figure 2.5).
Figure 2.3 Frontal view of facial development during the 5th and 6th week. Nasal placodes are elevated into horseshoe-shape, and later become the medial and lateral nasal processes. The medial nasal processes fuse with each other to form the philtrum, alveolar process, maxillary incisors, and primary palate. The lateral nasal processes develop into the alae of the nose. The mandibular processes fuse with each other to form the mandible and lower lip (adapted from Carlson, 2004).

Figure 2.4 Hard palate formation during the 6th week of embryonic life. Two lateral palatal shelves of the maxillary processes begin to grow (adapted from Carlson, 2004).
Figure 2.5 Mandible formation. A, During the 4th week, the two mandibular processes are separated from each other. B, During the 6th week, the two mandibular processes are fused with each other (adapted from www.google.com).

At the beginning of the 7th week, the tongue begins to develop. The two palatal shelves grow downward along the sides of the tongue. During the 8th week, the tongue moves downward, following the growth of the mandible, and subsequently the palatal shelves are elevated to the horizontal position, grow and fuse to each other during the 9th week (Figure 2.6-2.8).

Figure 2.6 Frontal view of facial development during the 7th and 8th weeks. The medial nasal, lateral nasal, maxillary and mandibular processes grow to contact and to fuse (adapted from Carlson, 2004).
Figure 2.7 Hard palate formation during the 7th week of embryonic life. The two lateral palatal shelves of the maxillary processes grow downward along the sides of the tongue. A, Horizontal view. B, Frontal view (adapted from Carlson, 2004).

Figure 2.8 Hard palate formation during the 8th week of embryonic life. The two lateral palatal shelves are elevated into horizontal position. A, Horizontal view. B, Frontal view (adapted from Carlson, 2004).

By the end of 8th week, palatal shelves contact horizontally with each other and with the primary palate anteriorly. Fusion of the palatal shelves commences, from anterior to posterior, but is not complete until the 11th week. Simultaneously with the fusion of the secondary palate, the nasal septum grows downward and fuses to the palate superiorly in the midline. The two nasal cavities are then completely separated (Carlson, 2004) (Figure 2.9).
Molecular control of secondary palate formation

Molecular control of palatal shelf growth have several networks operating between the palatal epithelium and the underlying mesenchyme during palatogenesis. These networks include signalling molecules and growth factors, such as Sonic hedgehog (Shh), members of the transforming growth factor β (Tgfβs) superfamily [including bone morphogenetic proteins (Bmps) and Tgfβs], fibroblast growth factors (Fgfs), their receptors, effectors and targets (Gritli-Linde, 2007). Transcription factors play roles in tissue patterning, growth and differentiation. Msx1 (Zhang et al., 2002), short stature homeobox Shox2 (Yu et al., 2005) and odd-skipped relate2 (Osr2) genes (Lan et al., 2004) have been shown to be expressed in developing palatal shelves.

Signaling pathways, involving Bmp4, Msx1, Shh and Bmp2, are necessary for normal growth and fusion of palatal shelves in the mouse (Zhang et al., 2002). Msx1
is localized in the anterior palatal mesenchyme. An autoregulatory loop involving Msx1 and Bmp4 induces expression of Shh in the medial edge epithelium of the palatal region. Epithelial Shh induces Bmp2 in the underlying anterior mesenchyme, and both Shh and Bmp2 regulate cellular proliferation of the anterior palate (Zhang et al., 2002). At embryonic day 12.5 (E12.5), Bmp4 and Bmp2 are expressed in the epithelium and mesenchyme of the developing palate. At E13.5, expression of Bmp2 remains unaltered. Bmp4 is found only in the anterior mesenchyme beneath the medial edge epithelium. Msx1 is strictly expressed in mesenchyme at E12.5 and E13.5. In Msx1−/− mice, alteration of mesenchyme proliferation may cause cleft palate (Zhang et al., 2002). Shh is expressed in the anterior and posterior palate. Its expression is localized in the medial edge epithelium of the anterior palate at E12.5 and E13.5, and in the oral epithelium of the posterior palate (Zhang et al., 2002). Shh has been shown to stimulate palatal shelf mesenchymal proliferation in vitro (Rice et al., 2004).

Osr2−/− mice develop cleft palate as a result of abnormal proliferation of palatal mesenchyme and delayed elevation of palatal shelves (Lan et al., 2004). It has been demonstrated that mice lacking Shox2 (Shox2−/−) have clefts of the anterior palate, a very rare type of palatal clefting (Yu et al., 2005), rarely found in humans or other mammals (Schüpbach, 1983). p63 is a homologue of p73, as well as a prototypic tumor suppressor of gene p53. p63 plays a role in epithelial differentiation and development. It regulates an array of factors that are essential for cell proliferation, integrity, and survival (Mills et al., 1999; Yang et al., 1999; Koster et al., 2004; Carroll et al., 2006). Mutations of p63 genes may cause at least five different ectodermal dysplasia syndromes, including ectrodactyly-ectodermal dysplasia and

During the 6th and 7th weeks of embryonic life, TBX22 is expressed in the developing palatal shelves (Braybrook et al., 2001; 2002). In the developing mouse palate, Tbx22 has been shown to be restricted posteriorly in a region encompassing both the soft palate and the posterior-most hard palate (Hillard et al., 2005). It has been reported that cleft palate and ankyloglossia syndrome is caused by mutations in TBX22 (Braybrook et al., 2001; 2002; Marçano et al., 2004; Chaabouni et al., 2005). Mutations in TBX22 have been reported to be associated with non-syndromic cleft palate (Marçano et al., 2004; Suphapeetiporn et al., 2007).

Subsequent to vertical growth, palatal shelves are elevated into a horizontal position. Additional growth allows contact between two opposing palatal shelves. Some genetic disruptions have been described to affect the growth of the palatal shelves at this stage. For instance, mice lacking the β3 subunit of the GABA_A receptor, or lacking Gad67, develop cleft palate without other craniofacial malformations (Culiat et al., 1993; 1995; Homanics et al., 1997). It has been reported that there are significant associations between GABRB3 (Scapoli et al., 2002) and GAD1 (Kanno et al., 2004) and non-syndromic cleft lip and/or palate. Delayed palatal shelf elevation occurs in Osr2^{−/−} (Lan et al., 2004) and Pdgfc^{−/−} (Ding et al., 2004).

Further growth of palatal shelves is essential for achieving midline contact of the medial edge epithelium (Figure 2.10). Failure at this stage interferes with successful
palatogenesis. The medial edge epithelium of the palatal shelves is composed of two layers. The inner layer is a basal layer of cuboidal cells lying on basement membrane. The outer layer is a flat-cell layer of periderm. Periderm cells gradually peel out from the epithelium surface. Some unpeeled peridermal cells are trapped between opposing shelves. Some trapped cells may die (Fitchett and Hay, 1989; Nawshad et al., 2004), but some may migrate into the nasal or oral epithelium (Dudas et al., 2007).

Cells of the basal layer forming the palatal seam may disappear by apoptosis (Martinez-Alvarez et al., 2000; Cuervo et al., 2002; Holtgrave and Stoltenburg-Didinger, 2002; Martinez-Alvarez et al., 2004), migration (Carette and Ferguson, 1992; Bittencourt and Bolognese, 2000), transformation into mesenchyme (Shuler et al., 1991; 1992; Martinez-Alvarez et al., 2000), or combination of all these mechanisms (Schupbach and Achroeder, 1983; Takigawa and Shiota, 2004).

TGFβs and their receptors play important roles during palatal fusion. During mouse palatogenesis, both Tgfβ1 and Tgfβ3 are expressed in the medial edge epithelium of the growing and fusing palatal shelves. Tgfβ2 is expressed only in the mesenchyme beneath the medial edge epithelium (Fitzpatrick et al., 1990; Pelton et al., 1990). Tgfβ1−/− mice do not develop cleft palate (Shull et al., 1992; Kulkarni et al., 1993), whereas Tgfβ2−/− mice have a relatively low incidence of cleft palate (Sanford et al., 1997). In contrast, Tgfβ3−/− mice exhibit cleft palate with 100% penetrance (Kaartinen et al., 1995; Proetzal et al., 1995). Cleft palate from Tgfβ3−/− mice is caused by failure of the fusion of the palatal shelves (Brunet et al., 1995; Taya et al., 1999). Tgfβ3 is required for the adhesion and fusion of the palatal shelves, probably by enhancing the transformation of the medial edge epithelium cells and inducing apoptosis in the medial edge epithelium (Kaartinen et al., 1997; Martinez-Alvarez et al., 2000).
Mutations in TGFβ3 have been associated with several cases of isolated cleft palate in humans (Lidral et al., 1998).

Figure 2.10 Diagrams summarizing the possible fates of the cells forming the palatal midline seam. A, Pre-fusion palatal shelves are composed of mesenchyme (green), covered by two-layered epithelium comprising a basal layer of cuboidal cells (light blue), and a superficial layer of flat cells (periderm, dark blue). B, Peridermal cells slough, some may die (red), but some may migrate into the oral or nasal epithelium. C, Some peridermal cells may become trapped between the two apposing shelves. D, Basal cells forming the palatal seam may disappear by apoptosis, migration, or transdifferentiation to mesenchyme, or through any possible combination of any of these processes. E, Following midline seam disappearance, apposed palatal shelves begin fusion (adapted from Dudas, 2007).

2.1.5 Clinical problems of cleft lip with or without cleft palate

Structures surrounding the defects may be associated with deformations or dysfunctions, such as feeding difficulties, speech disorders, ear disease, respiratory difficulties, dental problems, and psychological problems.
2.1.5.1 Feeding difficulties

Patients with cleft palate usually have some difficulties in performing bottle or breast feeding, and/or swallowing difficulties because food does not follow the normal swallowing pathway. Clefts of the palate do not allow infants to create negative pressure upon sucking (Richard, 1991).

2.1.5.2 Speech disorder

Abnormal articulation is common in patients with cleft palate. Velopharyngeal incompetence is associated with an audible escape of air from the nose during production of pressure sounds. About 75% of patients after primary palate surgery have velopharyngeal competence. Velopharyngeal competence is the most important articulatory performance for the speech of patients with cleft palate (Persson et al., 2006).

2.1.5.3 Ear diseases

Patients with an isolated cleft lip have an incidence of hearing loss similar to that of the normal population, but the incidence of hearing loss in patients with cleft palate is higher. The most common ear diseases are Eustachian tube dysfunction and conductive hearing loss. Eustachian tube dysfunction is a result of abnormal insertion of levator and tensor veli palatini muscles into the posterior margin of the hard palate. The incidence of Eustachian tube dysfunction decreases with increasing age. The Eustachian tube connects the middle ear to the back of the nose, and functions to equalize air pressure between these two areas. An abnormal Eustachian tube causes imbalance of air pressure and causes mucus production from the lining of the middle ear. This mucus reduces the transmission of sound through the middle ear, resulting
2.1.5.4 Respiratory difficulties

Cleft lip and/or palate may cause problems with the upper airway or with breathing. Cleft lip and palate may produce nasal deformities and reduce the size of the nasal airway. Patients with Pierre Robin sequence have airway problems as a result of mandibular hypoplasia, glossoptosis, and cleft of the secondary palate (Hocevar-Boltezar, 2006).

2.1.5.5 Dental problems

Dental anomalies in patients with clefts include hypodontia, malformation, abnormal eruption pattern, displaced teeth, supernumerary teeth, and crowding. Enamel may have poor maturation, which leads to decay. The most frequent oral anomaly is congenital absence of one or more permanent teeth. In addition, hypodontia outside the cleft region is found in 30% of children with clefts, both cleft lip and palate and cleft palate only. It is more likely to occur in subjects with cleft lip and palate than in those with cleft lip only or with cleft palate only (Slayton et al., 2003).

2.1.5.6 Psychological problems

Initial emotional reactions from parents may be shock, confusion, grief, and guilt. They feel shy, embarrassed, or socially inhibited. Children with cleft lip and palate may perceive negative reaction from their parents at an early age. An imperfect appearance may initiate unwanted questions and reactions that induce feelings of
inferiority (Turner et al., 1998).

2.2 Ankyloglossia

Ankyloglossia or tongue-tie is a congenital anomaly characterized by an abnormally short lingual frenum, which variably restricts the movement of the tongue (Lalakea and Messner, 2003). It appears in various types (Figure 2.11).

![Figure 2.11 Various appearances of ankyloglossia.](image)

2.2.1 Classification of ankyloglossia

A classification of ankyloglossia has been developed by Kotlow (1999). This classification measures “free tongue” which is defined as the tongue’s length from the insertion of the lingual frenum from base to tip of tongue. According to Kotlow (1999), ankyloglossia is classified into five types.

1. Clinically acceptable, normal range of free tongue: greater than 16 mm.
2. Class I: Mild ankyloglossia: 12 to 16 mm.
3. Class II: Moderate ankyloglossia: 8 to 11 mm.
4. Class III: Severe ankyloglossia: 3 to 7 mm.

5. Class IV: complete ankyloglossia: less than 3 mm.

2.2.2 Incidence of ankyloglossia

Ankyloglossia in newborns occurs from 0.02% to 4.8%, and is more common in boys than girls. Male to female ratio is about 3 to 1. There are no significant differences among races (Messner and Lalakea, 2000; Ballard et al., 2002). In the majority of cases, ankyloglossia is an isolated condition, and its prevalence has been reported to be 10.4% in infants with a history of maternal cocaine abuse (Harris et al., 1992).

2.2.3 Embryogenesis of the tongue

The tongue arises in the ventral wall of the primitive oropharynx from the inner lining of the first to the fourth pharyngeal arches. During the 4th week in utero, mesenchyme in the internal aspect of the first pharyngeal arch develops into the anterior two thirds, or body, of the tongue. Median mesoderm in the ventral parts of the second, third, and fourth pharyngeal arches develops into the posterior third or root of the tongue. The posterior part of the fourth pharyngeal arch develops into the epiglottis (Figure 2.12).

During development, the tongue is fused to the floor of the mouth. Separation of the tongue and the floor of the mouth is achieved by extensive cellular degeneration (Carlson, 2004). The frenum is the only remaining tissue in this area (Stevenson and Hall, 2006).
Figure 2.12 Frontal view of the internal wall of the primitive pharyngeal cavity during embryonic tongue development. A-C, Mesenchyme in the ventral wall of the first pharyngeal arch (green) is swelling to form the anterior two thirds of the tongue. Mesoderm of the ventral parts of the second, third, and fourth pharyngeal arches (blue) swells to form the copula, or hypobranchial eminence, which develops into the posterior third, or root, of the tongue. The posterior part of the fourth arch (purple) swells and develops into the epiglottis (adapted from Carlson, 2004).

2.2.4 Consequences of having ankyloglossia

Restriction of tongue tip mobility results in various degrees of complication, such as sucking or feeding dysfunction in infants and children, speech problems, mechanical and social problems due to restriction of tongue movement, spacing of lower incisors, gingival recession, and malocclusion (Messner and Lalakea, 2000; Williams and Waldron, 1985).
2.2.4.1 Feeding difficulties

In a newborn child, ankyloglossia may result in breast feeding difficulties, including ineffective latch, inadequate milk transfer, and maternal nipple pain. An infant with ankyloglossia may experience difficulty latching on to the nipple, and may compress the nipple against the gum pad instead of the tongue, resulting in nipple pain and inadequate seal. A mother experiencing such pain may often contemplate switching her baby to bottle feeding (Kupietzky and Botzer, 2005; Lalakea and Messner, 2003).

2.2.4.2 Speech disorders

Some children with ankyloglossia develop normal speech and compensate for the limited tongue tip mobility with speech therapy. Some children may have problems with articulation. Speech sounds may be affected by impaired tongue tip mobility, including lingual sounds and sibilant sounds, such as “t”, “d”, “n”, “l”, “th”, “z”, and “s”. The patients who have difficulty producing these sounds accurately should be referred to a speech pathologist for evaluation (Kupietzky and Botzer, 2005; Lalakea and Messner, 2003; Messner and Lalakea, 2000).

2.2.4.3 Malocclusion

Ankyloglossia results in inability to raise the tongue to the roof of the mouth. This may prevent development of a normal adult swallowing pattern. Anterior tongue position or tongue thrusting during swallowing may cause anterior open bite. Restriction of free upward and backward movement of the tongue may result in an exaggerated anterior thrusting of the tongue against the anterior body of mandible,
and this produces mandibular prognathism (William and Waldron, 1985).

2.2.5 Ankyloglossia-associated syndromes

Ankyloglossia can be a part of genetic syndromes such as Van Der Woude syndrome, Kindler syndrome and Cleft palate with ankyloglossia.

2.2.5.1 Van Der Woude syndrome

Van Der Woude syndrome (VWS; OMIM 119300) is characterized by pits and/or sinuses of the lower lip, and cleft lip and/or cleft palate. Other less frequent features include sensorineural hearing loss, prominent frontal bone, large frontal/sphenoid/maxillary sinus with increased mastoid air cells, long tooth roots, dental pulp stones, ankyloglossia, brachydactyly of the hands, brachyphalangy and hyperphalangy of toes (Burdick et al., 1987; Kantaputra et al., 2002).

The gene responsible for Van Der Woude syndrome is interferon regulatory factor-6 (IRF-6) which is located on chromosome 1q32-q41. Van der Woude syndrome and popliteal pterygium syndrome (PPS) are caused by mutations of IRF6. Popliteal pterygium syndrome has all the features of Van Der Woude syndrome, plus popliteal pterygium, syngnathia, toe/nail abnormality, syndactyly, and genito-urinary malformations (Kondo et al., 2002).

2.2.5.2 Kindler syndrome

Kindler syndrome is characterized by vesiculopapular lesions of the hands and feet, reticulate erythema, diffuse continuous atrophy, poikiloderma, limitation of mouth opening, ankyloglossia and atrophy of the buccal mucosa with white spots
(Hacham-Zadeh and Garfunkel, 1985). The genes responsible for Kindler syndrome are \textit{FERM} (Jobard \textit{et al}., 2003) and \textit{KIND1} (Siegel \textit{et al}., 2003).

### 2.2.5.3 Cleft palate with ankyloglossia

Cleft palate with ankyloglossia syndrome (CPX) was originally reported by Lowry in 1970. It is characterized by cleft palate and ankyloglossia. It is inherited as a semidominant, X-linked inheritance, producing an intermediate phenotype in the heterozygous condition. Affected males may have cleft palate only, ankyloglossia only, or cleft palate with ankyloglossia. Female carriers may have phenotypes ranging from cleft palate with ankyloglossia to asymptomatic. Approximately 70-80\% of female carriers have ankyloglossia only. Phenotypic variation within a family includes bifid uvula, high, arched palate, and ankyloglossia, either alone or in combination, especially in males (Moore \textit{et al}., 1988; Gorski \textit{et al}., 1992). The gene responsible for cleft palate and ankyloglossia syndrome is the transcription factor \textit{TBX22} gene. Complete loss of \textit{TBX22} function, secondary to nonsense, missense, frameshift, and splice site mutations, causes a range of phenotypes in affected males. Haploinsufficiency causes phenotypes in affected females (Braybrook \textit{et al}., 2001; Braybrook \textit{et al}., 2002; Marçano \textit{et al}., 2004; Chaabouni \textit{et al}., 2005; Suphapeetiporn \textit{et al}., 2007).

### 2.3 Hypodontia

Hypodontia is the most common developmental anomaly in humans. In its mild form, one or few teeth are absent, while in its severe form several teeth are absent. The prevalence of agenesis of permanent teeth, excluding third molars, ranges from
2.2% to 10.1% (Polder et al., 2004). Agenesis of primary teeth is rare. In Caucasian populations, it ranges from zero to 0.9% (Jarvinen and Lehtinen, 1981). Prevalence in Malaysian (Nik-Hussein, 1989), Hongkong (Davis, 1987), Japanese (Endo et al., 2006), and Thai (Dechkunakorn et al., 1990) populations are 2.8%, 6.9%, 8.5%, and 8.7%, respectively.

Hypodontia has no predilection between left and right sides. Hypodontia of the permanent dentition is not significantly different between maxilla and mandible. The incidence of hypodontia of individual teeth varies in frequency between the jaws, and hypodontia in the primary dentition is more common in the maxilla than in the mandible (Daugaard-Jensen et al., 1997).

2.3.1 Tooth development

The teeth develop from the mucosal epithelial lining of the oral cavity and cranial neural crest-derived ectomesenchymal cells. Extention of epithelial cells into the underlying ectomesenchyme to form the dental lamina is the beginning of tooth formation. The dental lamina determines the sites of future teeth, 20 for the deciduous dentition and 32 for the permanent one. The dental lamina, a bud-like thickening surrounded by ectomesenchymal cells, represents the earliest stage of the tooth germ (Figure 2.13).
Figure 2.13 Early stage of tooth formation. A, Oral epithelial thickening and underlying mesenchyme. B, Diagram of tooth formation in the bud stage (adapted from Slootweg, 2007).

Proliferation of oral ectoderm at specific tooth-forming sites along the future dental axis invaginates into the underlying ectomesenchyme to form tooth buds. This stage is induced by Sonic hedgehog (Shh), a signaling peptide that is localized in the oral ectoderm (Bitegood and McMahon, 1995; Cobourne et al., 2001). Sonic hedgehog has a reciprocal pattern of expression to Wnt7b, a member of the WNT family. Wnt7b is also expressed in non-dental oral epithelium. The overexpression of Wnt7b in dental epithelium leads to downregulation of Shh expression, and an arrest of tooth development at the ectodermal thickening stage (Tucker and Sharpe, 2004).

In the underlying mesenchyme, the position of the tooth fields seems to be occupied by the paired box gene Pax9. Pax9 is the earliest mesenchymal marker of the positions of the presumptive tooth germs. Along with Barx1, Pax9 is positively regulated by FGF8, and negatively regulated by BMP4, and also involves repression by another member of the bone morphogenetic family, Bmp2 (Tucker and Sharpe, 2004).

In the bell stage of tooth development, the epithelial cells of the enamel organ are comprised of the inner enamel epithelium layer lying over the dental papilla, and the outer enamel epithelium layer near the dental follicle. The area between the inner
and outer enamel epithelium contains loose stellate epithelium, which is called the stellate reticulum (Figure 2.14).

Figure 2.14 Middle stage of tooth formation. A, Cap stage of tooth development. B, Bell stage of tooth development (adapted from Slootweg, 2007).

2.3.2 Molecular mechanisms underlying tooth formation

Tooth morphogenesis is directed through oral epithelial-mesenchymal interactions (Figure 2.15). Oral epithelium in the molar field strongly expresses fibroblast growth factors 8 and 9 (Fgf8 and Fgf9). The epithelium in the incisor field mainly expresses Bone Morphogenetic Protein 4 (BMP4). These signaling molecules control homeobox gene expression in underlying neural crest-derived mesenchyme. FGF8 (and to a lesser extent, FGF9) positively regulates expression of Barx1 (BarH-like homeobox 1) and Dlx2 (distal-less homeobox 2), while Bmp4 positively regulates expression of Msx1 and Msx2, but simultaneously negatively regulates expression of Barx1. This results in restriction of Barx1 and Dlx2 to the presumptive molar region, and Msx1 and Msx2 to the presumptive incisor region. In rostral–caudal pattern, Fgf8 signalling turns on expression of LIM homeobox genes Lhx6 and Lhx7. The homeobox gene Gsc (goosecoid) is expressed after Lhx6/7, and is also positively regulated by FGF8 (Tucker and Sharpe, 2004).
Figure 2.15 Pattern of gene expression in developing tooth at embryonic day (E) 10.5. The diagram shows an isolated mandibular arch. Auto-regulatory loops within oral epithelium and underlying mesenchyme lead to formation of strict boundaries of gene expression, which set up presumptive incisor and molar fields. \textit{Bapx1}, bagpipe homeobox gene 1 homologue; \textit{Barx1}, BarH-like homeobox 1; \textit{Dlx}, distalless homeobox; \textit{Gsc}, goosecoid; \textit{Lhx}, LIM homeodomain genes; \textit{Msx}, homeobox, msh-like 1; \textit{Pitx}, paired-related homeobox gene (adapted from Tucker and Sharpe, 2004).

After the Bud stage, tooth germ epithelium invaginates around the condensing mesenchyme, and a structure known as the primary enamel knot is created at its center, forming the Cap stage of tooth development. The enamel knots express many signalling factors, such as \textit{SHH} and members of the \textit{FGF}, \textit{BMP} and \textit{WNT} families. In teeth with many cusps, after disappearance of the primary enamel knot at the late Cap stage, secondary enamel knots are formed at the sites of future cusp tips within the internal enamel epithelium. These secondary knots regulate the formation of the Bell stage tooth germ (Tucker and Sharpe, 2004).
Interaction between epithelium and mesenchyme causes cells of the inner enamel epithelium to transform into ameloblasts. The inner enamel epithelium induces differentiation of the outer cells of the dental papilla into odontoblasts; therefore, dentin is produced before enamel. The odontoblasts form a matrix of collagen fibers, which, after calcification, becomes dentin (Figure 2.16). Ameloblasts form the enamel matrix subsequent to the formation of a tiny amount of dentin. After crown formation, the inner enamel epithelium transforms into the epithelial root sheath of Hertwig. This transformation induces differentiation of the dental papilla into odontoblasts to form root dentin.

![Figure 2.16](image_url)

**Figure 2.16** Late stage of tooth formation. A, Deposition of enamel (purple) and dentin (pink) at the tip of the dental papilla. B, Root formation by cells from dental papilla transform into odontoblasts (adapted from Slootweg, 2007).

Ectomesenchymal cells from the dental follicle differentiate into cementoblasts, which form cementoid, covering the root surface. After that, cementum is formed by calcification of cementoid. Remnants of Hertwig’s root sheath become a component of the periodontal ligament, and play a role in preventing contact between the root surface and the alveolar socket (Slootweg, 2007). Tooth formation starts around the 6th week of embryonic life, and continues until early adulthood.

Hypodontia is under the influence of the genetic mechanism involved in tooth
formation. Many reports show several genes responsible for hypodontia, such as MSXI (van den Boogaard et al., 2000; Lidral and Reising, 2002; Mostowska et al., 2006), and PAX9 (Stockton et al., 2000; Nieminen et al., 2001; Das et al., 2002; Lammi et al., 2003; Jumlongras et al., 2004; Klein et al., 2005; Kapadia et al., 2006). Hypodontia-associated syndromes include Rieger syndrome, Witkop syndrome, Hypohidrotic ectodermal dysplasia, Ectrodactyly-Ectodermal dysplasia and Cleft lip/palate (EEC), and Cleft lip/palate-Ectodermal dysplasia syndrome (CLPED1). They are caused by mutations in PITX2, MSXI, EDA/EDAR, P63, and PVRL1, respectively (Semina et al., 1996; Bayes et al., 1998; Suzuki et al., 2000; Thesleff, 2000; Jumlongras et al., 2001; van Bokhoven et al., 2001).

Tooth development consists of sequential and reciprocal signaling processes between epithelial and mesenchymal cell layers that are controlled by genes encoding growth factors, by extracellular matrix components and by transcriptional factors (Tucker and Sharpe, 2004).

2.3.3 Clinical problems associated with hypodontia

Hypodontia is prone to cause malocclusion, whose severity depends on the number of missing teeth. Hypodontia may affect craniofacial development, especially maxillary retrognathism and reduced anterior facial height (Mattheeuws et al., 2004; Nodal et al., 1994; Endo et al., 2006). It is often associated with reduction of tooth dimensions and morphology, delays of development, root anomalies, abnormal positions, and also enamel hypoplasia (Ahmad et al., 1998; Baccetti, 1998).
2.4 *TBX22* gene

T-box gene was initially identified in mice. Heterozygous mutations cause short tail while homozygous mutations are lethal in utero, and result in lack of notochord and mesoderm posterior to somite 7 (Dobrovolskaia-Zavadskaja, 1927). This gene family acts as transcription factors that share a DNA binding domain or T domain in the N-terminal region, which encodes approximately 200 amino-acids (Laugier-Anfossi and Villard, 2000). It binds to specific sequences on a DNA target gene. Mutations in highly conserved regions of other T-box genes cause human disorders, such as *TBX1* mutations in patients with DiGeorger syndrome (Zwier *et al*., 2007), *TBX3* mutations in patients with Ulnar-mammary syndrome (Sasaki *et al*., 2002) and *TBX5* mutations in patients with Holt-Oram syndrome (Heinritz *et al*., 2005). In a recent study, *Tbx22* knockout mice had submucous cleft palate, ankyloglossia, and, in some mice, choanal atresia (Pauws *et al*., 2009).

2.4.1 *TBX22* structure

The *TBX22* sequence is mapped at chromosome Xq12-q21. It is composed of 8 exons spanning 8.7 kilobases of genomic DNA (Figure 2.17). *TBX22* has 9,487 nucleotides, which encode a 400-amino-acid protein, and contains a T domain in the NH$_2$-terminal region (Laugier-Anfossi and Villard, 2000) (Figure 2.18).

![Figure 2.17 Genomic structure of *TBX22*. The T domain is in the gray box (from exon 2 to exon 6).](image-url)
Figure 2.18 Sequence of TBX22 cDNA. Codons are presented individually in an open reading frame and corresponding amino acids are indicated using a single letter code below each codon. The first in-frame stop codon in the 5’ untranslated region is underlined. The polyadenylation site in the 3’ untranslated region is doubly underlined. Solid vertical lines show positions of exon–intron boundaries. The T-domain coding region is shaded (adapted from Laugier-Anfossi, 2000).
2.4.2 *TBX22* expression

*TBX22* is expressed during palatogenesis in human development (Figure 2.19-2.20). It is first detected as a weak signal in the 1st pharyngeal arch at around 37 days post ovulation. At nearly 41 days post ovulation, *TBX22* is strongly expressed in the mesenchyme of the lateral and medial nasal processes, the lateral palatal processes, and the base of the tongue. At about 48 days post ovulation, it is expressed in the mesenchyme of the developing face, base of brain, developing nose, and primary palate. It is strongly expressed in the developing lateral palatal processes and the oronasal membrane. At 50 to 52 days post ovulation, its strong expression is detected in the vertical palatal shelves. Expression is stronger in the medial parts than in the lateral parts. At 56 days post ovulation, it is expressed in the horizontal palatal shelves, which are still separate. Expression is found at the base of the tongue (prospective lingual frenum), the base of developing nasal septum, the mesenchyme surrounding the developing nostrils, and the mesenchyme surrounding the developing eyes. In the 9-week fetus, the palatal shelves have made contact. *TBX22* is undetectable in the palatal shelves, but is still expressed in the mesenchyme surrounding the developing nostrils, in the nasal cartilage, and in the tongue.
Figure 2.19 *TBX22* expression. A, C, E, G, I and K are bright-field images of haematoxylin and eosin (H&E)-stained sections, while B, D, F, H, J and L are dark-field images of adjacent sections that have been hybridized with an antisense TBX22 RNA probe. A and B, Transverse sections from a CS17 embryo (41 days post ovulation); C and D, Sagittal sections from a CS19 embryo (48 days post ovulation). E–J, Transverse sections from a CS19 embryo. K and L, Transverse sections from a CS20 embryo (50–51 days post ovulation). The dotted lines in C represent the approximate plane of section in the corresponding panels. Bar = 400 µm; e, eye; lpp, lateral palatal process; l, lateral nasal process; m, medial nasal process; o, oronasal membrane; ps, palatal shelf; t, tongue (adapted from Braybrook *et al.*, 2002).
Figure 2.20 TBX22 is expressed in the developing nasal septum but not in the fused palatal shelves. A, C, E and G, bright-field images of H&E-stained sections. B, D, F and H, dark-field images of adjacent sections that have been hybridized with an antisense RNA probe. A and B, Transverse sections from a CS21 embryo (52 days post ovulation). C and D, Frontal sections from a CS23 embryo (56 days post ovulation). E–H, Frontal sections of a fetus at 9 weeks of development. Bar = 1000 µm; e, eye; nc, nasal cartilage; no, nostrils; ns, nasal septum; om, odontogenic mesenchyme; ps, palatal shelf; t, tongue (Adapted from Braybrook et al., 2002).

There is strong expression in the odontogenic mesenchyme of developing tooth buds (Braybrook et al., 2002). Tbx22 expression in the mouse showed that it was also expressed in developing lung epithelium and in developing whiskers (Braybrook et al., 2002) (Figure 2.21). Other sites of expression, as studied in chick ortholog of TBX22, are limb and cranial mesenchyme (Haenig et al., 2002) (Figure 2.22).
Figure 2.21 *Tbx22* expression in developing lung epithelium and in developing whiskers of a developing mouse (Braybrook *et al*., 2002).

Figure 2.22 Whole-mount, *in situ*, hybridization analysis of *Tbx22* expression during chick development. Blue arrowhead indicates expression in mesenchyme of proximal region of forelimbs (Haenig *et al*., 2002).

2.4.3 TBX22 function

TBX22 protein is a transcription factor that controls the activity of other genes. It plays essential roles during early development in cell type specification and regulation of morphogenic movement (Wilson and Colon, 2002; Tada and Smith, 2001). The original T-box gene (Brachyury) binds to a palindromic sequence (T[G/C]ACACCTAGGTGAAATT) as a dimer (Kispert and Herrmann, 1993) and can bind to a half palindromic sequence (T/C)TTCACCT (Casey *et al*., 1998). All T-box proteins can bind to Brachyury target sequences (Tada *et al*., 1998; Carreira *et
al., 1998; Hsueh et al., 2000). Examples of genes that contain variations of Brachyury-binding site sequences are members of the transforming growth factor (TFGβ) and epidermal growth factor (EGF) families, paraxial protocadherin (Papc), and Brachyury-induced homeobox (Bix) genes. One possible target gene of Tbx22 is Tfgb3. Tfgb3 knockout mice have cleft palate with delayed pulmonary development (Karrtinen et al., 1995). TFGβ3 expression during palatal fusion supports its important role in the development of the palate (Fitzpatrick et al., 1990; Karrtinen et al., 1997). Tgfb3 and its receptor (Tbr-II) are expressed in oral epithelial cells and are most strongly expressed in the medial edge epithelial cells of the palatal shelves (Cui et al., 1998). Tbx22 and Tgfb3 have an overlapped expression pattern. Tgfb3 is expressed very strongly in medial edge epithelial cells, while Tbx22 is predominantly expressed in the mesenchyme underneath the medial edge epithelial cells. The epithelial-mesenchymal interaction induces palatal development (Ferguson and Honig, 1984).

TBX22 has both repression and activation domains. Transcriptional repression can be autoregulated through distal TBX22 promoter, and can be regulated with small ubiquitin-like modifier protein (SUMO). SUMO1 attaches to K63 within the N-terminal region, which contains lysine. This attachment is an absolute requirement for repressive activity. The disability of the DNA binding domain to conjugate with SUMO1 has a major effect on the regulation of downstream target interaction (Andreou et al., 2007). SUMO1 haploinsufficiency induces cleft lip and palate (Alkuraya et al., 2006).

2.4.4 Genotype-phenotype correlation

Previous studies have reported various TBX22 mutations in patients with cleft
palate with or without ankyloglossia (Figure 2.23). Those mutations consist of missense, nonsense, frameshift, and splice site mutations (Braybrook et al., 2001; Braybrook et al., 2002; Marçano et al., 2004; Chaabouni et al., 2005, Suphapeetiporn et al., 2007). With similar genetic backgrounds, affected patients demonstrate phenotypic variability. Affected males have shown evidence of cleft palate or a microform of cleft palate in 96% (79% for cleft palate with ankyloglossia and 17% for cleft palate only). Cleft palate phenotype ranges from complete cleft of the secondary palate, submucous cleft, bifid uvula, absent tonsils, to high arch palate. Ankyloglossia is found in 83% of males who had mutations in TBX22 (79% for cleft palate with ankyloglossia and 4% for ankyloglossia only). In carrier females, ankyloglossia only is a common phenotype (45%). Cleft palate is seen in 17% of female carriers (11% for cleft palate with ankyloglossia and 6% for cleft palate only). Nonpenetrance is found in nearly 38% (Marçano et al., 2004). Interestingly, TBX22 is expressed in developing tooth buds and in the base of the tongue, but a dental phenotype has not been described in patients with TBX22 mutations, and TBX22 mutations have not been found in patients with isolated ankyloglossia.

![Figure 2.23 Pathologic variants of TBX22 from previously reports.](image)