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REVIEW

Molecular basis of cleft palates in mice

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Abstract

Cleft palate, including complete or incomplete cleft palates, soft palate clefts, and submucosal cleft palates, is the most frequent congenital craniofacial anomaly in humans. Multifactorial conditions, including genetic and environmental factors, induce the formation of cleft palates. The process of palatogenesis is temporospatially regulated by transcription factors, growth factors, extracellular matrix proteins, and membranous molecules; a single ablation of these molecules can result in a cleft palate *in vivo*. Studies on knockout mice were reviewed in order to identify genetic errors that lead to cleft palates. In this review, we systematically describe these mutant mice and discuss the molecular mechanisms of palatogenesis.

Key words: Tbx1; Submucosal cleft palate; Incomplete cleft palate; Palatal shelf; Palatogenesis; Knockout mice

Core tip: Cleft lip and/or palate is one of the most frequent congenital craniofacial anomalies observed. Multifactorial conditions, including genetic and environmental factors, induce the formation of cleft palates. We screened knockout mice with cleft palate phenotypes and observed approximately 180 mice with the anomaly. In order to understand the molecular regulatory mechanisms of palatogenesis and to identify genetic errors that lead to cleft palates, we aimed to review studies performed using knockout mice with cleft palates.

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INTRODUCTION

Cleft lip and/or palate (CL/P) is the most frequent congenital craniofacial anomaly observed in humans, with an incidence of 1 per 700 births worldwide[1]. Furthermore, 55% of the patients with CL/P are reported to have a multiple malformation syndrome[2]. CL/P involves a multifactorial etiology, both genetic and environmental. Teratogens that cause CL/P in humans include common environmental exposures, such as alcohol, smoking, infections, dioxin, estrogen, retinoic acid, and altitude (reviewed by Murray[1]). The offspring of parents with CL/P present a higher incidence of CL/P than those without a family history[1]. Gene-environment interactions for non-syndromic CL/P have also been reported[1]. Cleft palate (CP) cases include complete CP, incomplete CP, and soft palate clefts. The mildest form of cleft palates is the soft palate cleft or bifid uvula because the initial palatal fusion occurs in the anterior region of secondary palatal shelves. Incomplete CP and soft palate clefts can manifest together with submucosal CP. This review focuses on studies performed using knockout mice with CP, aiming to clarify the molecular regulatory mechanisms of palatogenesis and to identify genetic errors underlying mammalian cleft palates.

MAMMALIAN PALATOGENESIS

The palate is formed with the primary and secondary palate. The primary palate is derived from the frontonasal prominence and becomes a small anterior part of the adult hard palate. The secondary palatal shelves extend bilaterally from the internal aspects of the maxillary prominences and will become the adult hard and soft palates. The process of palatogenesis consists of several stages: palatal shelf formation, elevation, and midline fusion of the palatal shelves (Figure 1). The secondary palatal shelves develop between embryonic day (E) 11.5 and 12.5 in the mouse embryo (Figure 1A). At E13.5, the palatal shelves grow downward on each side of the tongue (Figure 1B). As the jaws develop, the tongue descends and the palatal shelves elevate to a horizontal position above the dorsum of the tongue (E14). Continuing their growth, the bilateral palatal shelves meet at the midline and fuse between E14.5 and E15.5 (Figure 1C).

The palatal shelves are composed of the neural crest-derived mesenchyme and ectoderm-derived epithelia, which cover the palatal mesenchyme (Figure 1D). Both elevation and fusion of the secondary palatal shelves occur in the midline from anterior to posterior. The secondary palatal shelves also fuse with the primary palate, separating the oral and nasal cavities. The anterior two-thirds of the palate forms the hard palate with neural crest-derived palatal bones (Figure 2A). The posterior one-third of the palate forms the bone-free soft palate and is involved in the palatopharyngeal sealing. Disruption at any stage of the formation, elevation, growth, or fusion of the secondary palatal shelves results in CP[3].

MOUSE MODELS FOR STUDYING THE MOLECULAR MECHANISMS OF PALATAL DEVELOPMENT

Major advances have been achieved regarding the molecular mechanisms that regulate palatal development using genetically engineered mice. Deletions in many genes of mice result in CP and the most frequent phenotype seen is complete CP (Figure 2B). Uniquely, *Tbx1*$^{-/-}$ mice present various phenotypes of CP[4], including complete CP (Figure 2B), incomplete CP (Figure 2C), and anterior CP (Figure 2D). Bone staining showed that some mice potentially had a submucosal CP (Figure 2G). These observations are in agreement with various CP phenotypes in humans.

In order to elucidate the molecular pathogenesis of CL/P, we conducted a literature search on PubMed (http://www.ncbi.nlm.nih.gov/pubmed) and the Mouse Genome Informatics (MGI) from the Jackson Laboratory (http://www.informatics.jax.org). The search was limited to knockout mice with CP and excluded the teratogen-induced CP (Table 1). We also investigated diseases/syndromes using the Online Mendelian Inheritance in Man (OMIM) (http://omim.org). Not all the molecules involved in cleft palates in mice are correlated to CL/P in human (Table 1). When genes in Table 1 were analyzed by biological function using BioCarta (http://www.biocarta.com) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Database (http://www.genome.jp/kegg/pathway.html), the transforming growth factor (TGF), hedgehog, Wnt, fibroblast growth factor (FGF), and mitogen-activated protein kinase (MAPK) signaling pathways were found to be critical in palatogenesis (Table 2). When genes were analyzed by molecular function using the PANTHER (Protein ANalysis THrough Evolutionary Relationships) database (http://pantherdb.org)[5], the most significantly enriched molecular function was the "transcription factor", especially the "homeobox transcription factors" (Table 2 and Figure 3).

To analyze mutant mice with cleft palates, the defects in palatal shelf development were divided into the following six categories (Table 3), which were modified from a previously published classification[3]. The first category is the failure of the palatal shelf formation. The gene mutations affect the initial development of the palatal shelf. The second one is the abnormal fusion of the palatal shelves and the mandible or tongue. Oral fusions between the palatal shelves and the tongue or mandible are rare. In *Tbx1* (T-box 1) knockout mice, the posterior part of the palatal shelves fuse to the mandible, inhibiting the

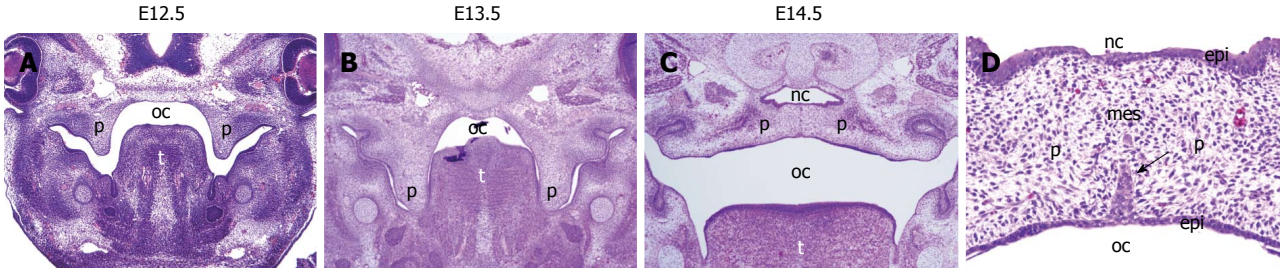

Figure 1 Palatogenesis in mice. Hematoxylin and eosin staining of coronal sections of the head of a wild-type mouse at embryonic day (E) 12.5 (A), E13.5 (B), and E14.5 (C, D). A: Mouse palatal shelves (p) develop from the maxillary prominences; B: By E13.5, the palatal shelves grow downward on each side of the tongue (t); C and D: At E14.5, the palatal shelves face each other along the midline above the tongue and fuse, separating the oral cavity (oc) from the nasal cavity (nc). The arrow in (D) indicates the medial edge epithelial (MEE) cells that constitute the midline epithelial seam. All animal experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committees of the University of Texas Southwestern Medical Center and Tokyo Medical and Dental University. mes: Mesenchyme; epi: Epithelium.

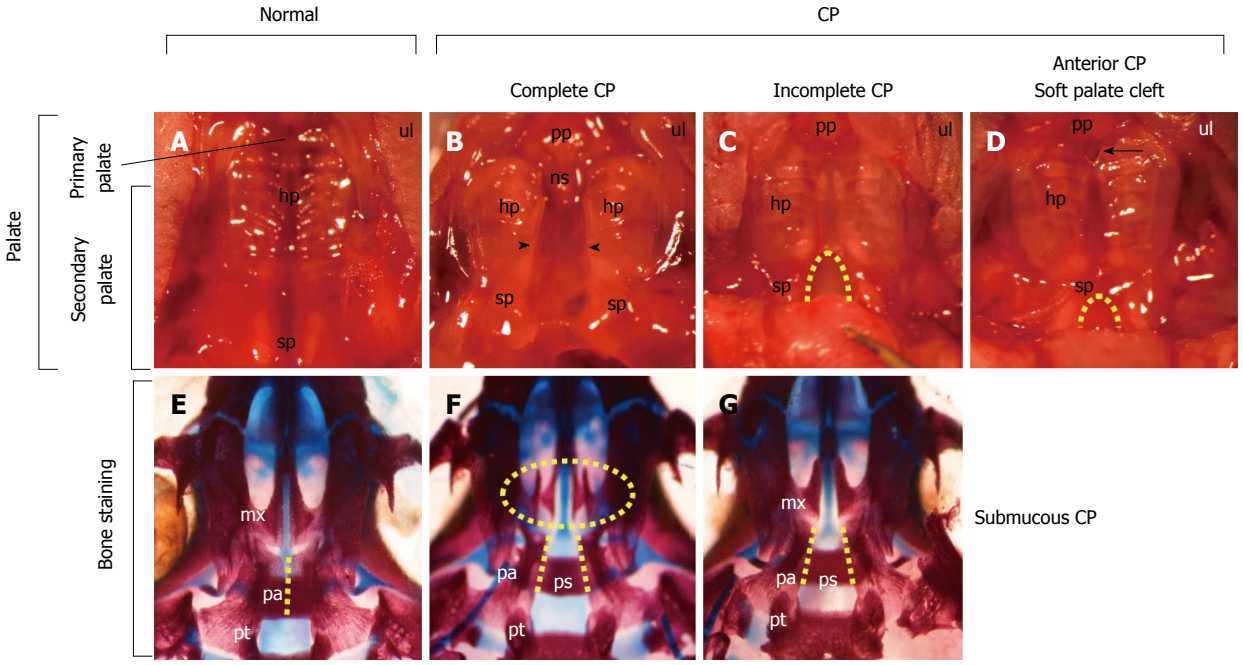

Figure 2 View of the palate from wild-type and *Tbx1*$^{-/-}$ mice with cleft palates. A-D: Ventral view of the maxilla of newborn wild-type (A) and *Tbx1*$^{-/-}$ mice with cleft palates (B-D). The palate consists of the primary palate (pp) and the secondary palate (sp), which consists of a hard palate (hp) and a soft palate (sp) (A). *Tbx1*$^{-/-}$ mice show complete cleft palate (CP) (arrowheads in B), incomplete CP (dashed line in C), and soft palate clefts associated with anterior CP (dashed line in D). An anterior CP (an arrow in D) is present at the junction between the primary palate and secondary palate, while the posterior palate remains fused; E-G: Ventral view of the cranial base of newborn wild-type (E) and *Tbx1*$^{-/-}$ mice (F, G) stained with alizarin red for mineralized bone and alcian blue for cartilage. Fusion of the bilateral palatal bones (pa) observed in the wild-type (dashed line in E) is absent in *Tbx1*$^{-/-}$ mice (dashed lines in F, G). The palatal shelves in the maxilla (mx) of *Tbx1*$^{-/-}$ mice with complete CP (oval dashed line in F) failed to grow toward the midline. Note the visible presphenoid bone (ps) associated with CP (F, G). Modified and used with permission from Funato *et al*[4]. ns: Nasal septum; pt: Pterygoid bone.

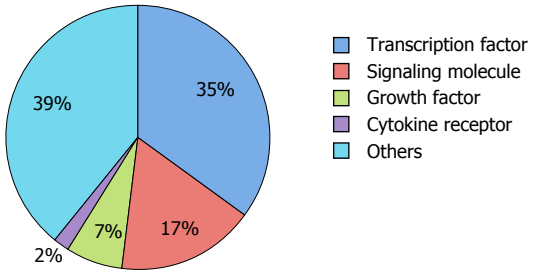

Figure 3 Gene ontology analysis of genes associated with cleft palate in mice. Gene ontology analysis of genes associated with cleft palate in mice was performed using the PANTHER classification system (http://pantherdb.org). The most significantly enriched molecular function was the "transcription factor" (P = 1.2 × 10^{-12}). A P value less than 0.05 was considered statistically significant.

elevation of the palatal shelves[4]. The third category is the failed or delayed palatal shelf elevation. Ablation of *Pax9* (paired box gene 9), *Pitx1* (paired-like homeodomain 1), *Gli2* (GLI family zinc finger 2), or *Osr2* (Odd-skipped related transcription factor 2) in the palatal mesenchyme results in the failed palatal shelf elevation[6-9], suggesting crucial roles for these transcription factors in controlling the mesenchymal cells during palatal shelf elevation. The fourth one is the failure of the palatal shelf development after elevation. The loss of *Msx1* (msh homeobox 1) and *Lhx8* (LIM homeobox gene 8) and the conditional ablation of *Tgfbr2* (transforming growth factor,

Table 1 Molecules involved in cleft palate in mice

Knockout mice with cleft palates			Humans		
Gene/category	**Protein**	**Ref.**	**OMIM**	**Syndrome**	**CL/P**
Growth factors, antagonist, and receptors					
Acvr1/Alk2 (*Wnt1-Cre*-mediated ablation)	Activin A receptor, type Ⅰ	[33]	[1]102576	Fibrodysplasia ossificans progressiva	nr
Acvr2a	Activin A receptor, type ⅡA	[34]	[1]102581	nr	nr
Bmp4	Bone morphogenetic protein 4	[35]	[1]112262	Microphthalmia, syndromic 6 Orofacial cleft 11	r
Bmp7	Bone morphogenetic protein 7	[36]	[1]112267	nr	nr
Bmpr1a/Alk3 (*Nestin-Cre*-mediated ablation)	Bone morphogenetic protein receptor, type ⅠA	[35]	[1]601299	Juvenile polyposis syndrome, Polyposis syndrome	nr
Chrd	Chordin	[37]	[1]603475	nr	nr
Ctgf	Connective tissue growth factor	[38]	[1]121009	nr	nr
Edn1	Endothelin 1	[39]	[2]131240	Auriculocondylar syndrome 3	r
Egfr	Epidermal growth factor receptor	[17]	[1]131550	nr	nr
Fgf9	Fibroblast growth factor 9	[40]	[1]600921	uc	nr
Fgf10	Fibroblast growth factor 10	[13,41]	[1]602115	Aplasia of lacrimal and salivary glands LADD syndrome	nr
Fgf18	Fibroblast growth factor 18	[42,43]	[1]603726	nr	nr
Fgfr1	Fibroblast growth factor receptor 1	[44]	[1]136350	Nonsyndromic cleft lip/palate Hartsfield syndrome Hypogonadotropic hypogonadism 2 Pfeiffer syndrome	r
Fgfr2 (knockout) (*Krt14-Cre*-mediated ablation)	Fibroblast growth factor receptor 2	[13,45]	[1]176943	Apert syndrome Crouzon syndrome Pfeiffer syndrome Saethre-Chotzen syndrome	r
Fst	Follistatin	[46]	[1]136470	nr	nr
Gabrb3	Gamma-aminobutyric acid A receptor, beta 3	[47]	[1]137192	Epilepsy, childhood absence, susceptibility to, 5	r
Gdf11/Bmp11	Growth differentiation factor 11	[48]	[1]603936	nr	nr
Gpr124	G protein-coupled receptor 124	[49]	[1]606823	nr	nr
Inhba	Inhibin, beta A/activin A	[50]	[1]147290	nr	nr
Pdgfc	Platelet-derived growth factor C	[51]	[1]608452	nr	r [52]
Pdgfra (knockout) (*Wnt1-Cre*-mediated ablation)	Platelet-derived growth factor receptor, alpha polypeptide	[53,54]	[1]173490	Gastrointestinal stromal tumor, somatic Hypereosinophilic syndrome, idiopathic, resistant to imatinib	r
Tgfb2	Transforming growth factor, beta 2	[55]	[1]190220	Loeys-Dietz syndrome, type 4	r
Tgfb3	Transforming growth factor, beta 3	[15,16,18]	[1]190230	Arrhythmogenic right ventricular dysplasia 1	r
Tgfbr1/Alk5 (*Wnt1-Cre*-, and *Nestin-Cre*-mediated ablation)	Transforming growth factor, beta receptor Ⅰ	[56,57]	[1]190181	Loeys-Dietz syndrome, type 1	r
Tgfbr2 (*Wnt1-Cre*-, and *KRT14-Cre*-mediated ablation)	Transforming growth factor, beta receptor Ⅱ	[12,58]	[1]190182	Loeys-Dietz syndrome, type 2	r
Vegfa	Vascular endothelial growth factor A	[59]	[2]192240	nr	nr
Membrane proteins					
Ceacam1	Carcinoembryonic antigen-related cell adhesion molecule 1	[60]	[1]109770	nr	nr
Efna5	Ephrin A5	[61]	[1]601535	nr	nr
Efnb1	Ephrin B1	[62]	[1]300035	Craniofrontonasal dysplasia	r
Efnb2	Ephrin B2	[63]	[1]600527	nr	nr
Fzd2	Frizzled class receptor 2	[64]	[1]600667	nr	nr
Itga5 (knockout) (*Mesp1-Cre*-mediated ablation)	Integrin alpha 5	[65,66]	[1]135620	nr	nr
Itgb1 (*Col2a1-Cre*-mediated ablation)	Integrin beta 1	[67]	[1]135630	nr	nr
Itgb8	Integrin beta 8	[68]	[1]604160	nr	nr
Jag1 (*Wnt1-Cre*-mediated ablation)	Jagged1	[69]	[2]601920	Alagille syndrome	nr
Jag2	Jagged2	[70]	[1]602570	nr	nr
Kcnj2	Potassium inwardly-rectifying channel, subfamily J, member 2	[71]	[1]600681	Andersen syndrome Atrial fibrillation, familial, 9 Short QT syndrome 3	r

Lrp6	Low density lipoprotein receptor-related protein 6	[72]	[1]603507	nr	nr
Ror2	Receptor tyrosine kinase-like orphan receptor 2	[73]	[1]602337	Robinow syndrome, autosomal recessive Brachydactyly, type B1	r
Ryk	Receptor-like tyrosine kinase	[74]	[1]600524	nr	nr
Ryr1	Ryanodine receptor 1, skeletal muscle	[75]	[1]180901	Central core disease King-Denborough syndrome Minicore myopathy with external ophthalmoplegia	nr
Sc5d/Sc5dl	Sterol-C5-desaturase (fungal ERG3, delta-5-desaturase) homolog (S. cerevisae)	[76]	[1]602286	Lathosterolosis	nr
Shh (*KRT14-Cre*-, and *Sox2-Cre*-mediated ablation)	Sonic hedgehog	[13,77]	[1]600725	Holoprosencephaly-3 Microphthalmia with coloboma 5 Single median maxillary central incisor	r
Smo/Smoh (*Wnt1-Cre*-mediated ablation)	Smoothened, frizzled class receptor	[78]	[1]601500	Basal cell carcinoma, somatic	nr
Tctn2	Tectonic family member 2	[79]	[1]613846	Meckel syndrome 8	r
Wls/Gpr177 (*Wnt1-Cre*-mediated ablation)	Wntless homolog (Drosophila)	[80]	[1]611514	nr	nr
Wnt5a	Wingless-type MMTV integration site family, member 5A	[81]	[1]164975	Robinow syndrome, autosomal dominant	r
Wnt9b (knockout) (*Foxg1-Cre*-mediated ablation)	Wingless-type MMTV integration site family, member 9B	[82,83]	[1]602864	nr	nr
Transcription and nucleolar factors					
Alx1	Aristaless-like homeobox 1	[84]	[1]601527	Frontonasal dysplasia 3	r
Alx3	Aristaless-like homeobox 3	[85]	[1]606014	Frontonasal dysplasia 1	r
Alx4	Aristaless-like homeobox 4	[85]	[1]605420	Frontonasal dysplasia 2 Parietal foramina 2 Craniosynostosis 5	Cleft alae nasi
Anp32b	Acidic (leucine-rich) nuclear phosphoprotein 32 family, member B	[86]	nr	nr	nr
Arid5	AT-rich interaction domain-containing protein 5A	[87]	[1]611583	nr	nr
Asxl1	Additional sex combs like 1	[88]	[1]612990	Bohring-Opitz syndrome Myelodysplastic syndrome, somatic	r
Barx1	BarH-like homeobox 1	[89]	[1]603260	nr	nr
Cdc42 (*Prrx1-Cre*-mediated ablation)	Cell division cycle 42	[90]	[1]116952	nr	nr
Chd7 (heterozygotes) (*Wnt1-Cre*-mediated ablation)	Chromodomain helicase DNA binding protein 7	[91,92]	[1]608892	CHARGE syndrome Hypogonadotropic hypogonadism 5 with or without anosmia	r
Cited2	CBP/p300-interacting transactivator, with Glu/Asp-rich C-terminal domain, 2	[93]	[1]602937	Atrial septal defect 8 Ventricular septal defect 2	nr
Crebbp/Cbp	CREB binding protein	[94]	[1]600140	Rubinstein-Taybi syndrome	nr
Dlx1	Distal-less homeobox 1	[95]	[1]600029	nr	nr
Dlx2	Distal-less homeobox 2	[95]	[1]126255	nr	nr
Dlx5	Distal-less homeobox 5	[96,97]	[1]600028	Split-hand/foot malformation 1 with sensorineural hearing loss	r
Dph1/Ovca1	DPH1 homolog (*S. cerevisiae*)	[98]	[1]603527	nr	nr
Eya1	Eyes absent 1 homolog (Drosophila)	[99]	[1]601653	Branchiootic syndrome 1 Branchiootorenal syndrome 1, with or without cataracts Anterior segment anomalies with or without cataract	r
Foxc2/Mfh1	Forkhead box C2	[100]	[1]602402	Lymphedema-distichiasis syndrome	r
Foxd3 (*Wnt1-Cre*-mediated ablation)	Forkhead box D3	[101]	[1]611539	uc	nr
Foxe1/Titf2/Fkhl15	Forkhead box E1	[102]	[1]602617	Bamforth-Lazarus syndrome Nonsyndromic orofacial clefting	r
Foxf2	Forkhead box F2	[103]	[1]603250	nr	nr
Gbx2	Gastrulation brain homeobox 2	[104]	[1]601135	nr	nr
Gli2	GLI family zinc finger 2	[8]	[1]165230	Culler-Jones syndrome Holoprosencephaly-9	r
Gli3	GLI family zinc finger 3	[105]	[1]165240	Greig cephalopolysyndactyly syndrome Pallister-Hall syndrome	r

Gsc	Goosecoid homeobox	[106]	[1]138890	Short stature, auditory canal atresia, mandibular hypoplasia, skeletal abnormalities	nr
Hand2/dHand	Heart and neural crest derivatives expressed 2	[107]	[1]602407	nr	nr
Hic1	Hypermethylated in cancer 1	[108]	[1]603825	nr	nr
Hoxa2	Homeobox A2	[19]	[1]604685	Microtia with or without hearing impairment (AD) Microtia, hearing impairment, and cleft palate (AR)	r
Irf6	Interferon regulatory factor 6	[109,110]	[1]607199	van der Woude syndrome Orofacial cleft 6 Popliteal pterygium syndrome 1	r
Jmjd6/Ptdsr	Jumonji domain containing 6	[111]	[1]604914	nr	nr
Kat6a/Moz/Myst3	K (lysine) Acetyltransferase 6A	[112]	[1]601408	nr	nr
Lhx7	LIM homeobox gene 7	[113]	nr	nr	nr
Lhx8	LIM homeobox gene 8	[11]	[1]604425	nr	r
Luzp1	Leucine zipper protein 1	[114]	[1]601422	nr	nr
Mef2c (*Wnt1-Cre*-mediated ablation)	MADS box transcription enhancer factor 2	[115]	[1]600662	Chromosome 5q14.3 deletion syndrome Mental retardation, stereotypic movements, epilepsy, and/or cerebral malformations	nr
Meox2	Mesenchyme homeobox 2	[116]	[1]600535	nr	nr
Mn1	Meningioma 1	[117]	[1]156100	Meningioma	nr
Mnt	Max binding protein	[118]	[1]603039	nr	nr
Msx1	Msh homeobox 1	[10,23]	[1]142983	Ectodermal dysplasia 3, Witkop type Orofacial cleft 5 Tooth agenesis, selective, 1, with or without orofacial cleft	r
Msx2 (missense mutation)	Msh homeobox 2	[119]	[1]123101	Craniosynostosis, type 2 Parietal foramina 1 Parietal foramina with cleidocranial dysplasia	r
Nabp2/Obfc2b/hSSB1	Nucleic acid binding protein 2	[120,121]	[1]612104	nr	nr
Osr2	Odd-skipped related transcription factor 2	[9]	[1]611297	nr	r
Pak1ip1	PAK1 interacting protein 1	[122]	[1]607811	nr	nr
Pax9	Paired box gene 9	[6]	[1]167416	Tooth agenesis, selective, 3	nr
Pbx1	Pre B cell leukemia homeobox 1	[83]	[1]176310	Leukemia, acute pre-B-cell	nr
Pds5a	PDS5, regulator of cohesion maintenance, homolog A (*S. cerevisiae*)	[123]	[1]613200	nr	nr
Phc1/Rae28	Polyhomeotic homolog 1	[124]	[1]602978	uc	nr
Pitx1	Paired-like homeodomain 1	[7,125]	[1]602149	Clubfoot, congenital, with or without deficiency of long bones and/or mirror-image polydactyly Liebenberg syndrome	r
Pitx2	Paired-like homeodomain 2	[126]	[1]601542	Axenfeld-Rieger syndrome, type 1 Iridogoniodysgenesis, type 2 Peters anomaly	nr
Pnn	Pinin	[127]	[1]603154	nr	nr
Prdm16	PR domain containing 16	[128]	[1]605557	Cardiomyopathy, dilated, 1LL Left ventricular noncompaction 8	nr
Prrx1/Prx1/Mhox	Paired related homeobox 1	[129]	[1]167420	Agnathia-otocephaly complex	r
Ptch1/Ptc1 (*Wnt1-Cre*-mediated ablation)	Patched 1	[130]	[1]601309	Basal cell nevus syndrome (Gorlin syndrome) Holoprosencephaly type 7	r
Pygo2 (*CMV-Cre*-mediated ablation)	Pygopus 2	[131]	[1]606903	nr	nr
Rax	Retina and anterior neural fold homeobox	[132]	[1]601881	Microphthalmia, isolated 3	nr
Recql4	RecQ protein-like 4	[133]	[1]603780	Baller-Gerold syndrome RAPADILINO syndrome Rothmund-Thomson syndrome	r
Runx2	Runt-related transcription factor 2	[134]	[1]600211	Cleidocranial dysplasia	r
Sall3	Spalt-like transcription factor 3	[24]	[1]605079	nr	nr
Satb2	SATB homeobox 2	[135,136]	[1]608148	Glass syndrome	r
Shox2	Short stature homeobox 2	[22]	[1]602504	nr	nr
Sim2	Single-minded family bHLH transcription factor 2	[137]	[1]600892	nr	nr

Smad4 (*Osr2-Cre*-mediated ablation)	SMAD family member 4	[138]	[1]600993	Juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome Myhre syndrome	nr
Smad7	SMAD family member 7	[139]	[1]602932	uc	nr
Snai2	Snail family zinc finger 2	[140]	[1]602150	Piebaldism Waardenburg syndrome, type 2D	nr
Sox5	SRY (sex determining region Y)-box 5	[141]	[1]604975	nr	nr
Sox9 (heterozygous) (*Wnt1-Cre*-mediated ablation)	SRY (sex determining region Y)-box 9	[142,143]	[1]608160	Acampomelic campomelic dysplasia	r
Sox11	SRY (sex determining region Y)-box 11	[144]	[1]600898	Mental retardation, autosomal dominant, 27	nr
Sp8	Sp8 transcription factor	[145]	[1]608306	nr	nr
Tshz1	Teashirt zinc finger family member 1	[146]	[1]614427	Aural atresia, congenital	nr
Tbx1 (knockout) (*KRT14-Cre*-mediated ablation)	T-box 1	[4,147]	[1]602054	DiGeorge syndrome Velocardiofacial syndrome Conotruncal anomaly face syndrome Tetralogy of Fallot	r
Tbx2	T-box 2	[148]	[1]600747	nr	nr
Tbx22	T-box 22	[149]	[1]300307	Cleft palate with ankyloglossia submucous cleft palate (SMCP)	r
Tcof1 (heterozygous)	Treacher Collins-Franceschetti syndrome 1	[150]	[1]606847	Treacher-Collins syndrome	r
Tfap2A (*Wnt1-Cre*-mediated ablation)	Transcription factor AP-2 alpha	[151]	[1]107580	Branchio-oculo-facial syndrome	r
Trp63/Tp63	Transformation related protein p63	[152]	[1]603273	Ectrodactyly, ectodermal dysplasia, and cleft lip/palate syndrome 3 Orofacial cleft 8 Hay-Wells syndrome Limb-mammary syndrome	r
Vax1	Ventral anterior homeobox 1	[153]	[1]604294	Microphthalmia, syndromic 11	r
Whsc1	Wolf-Hirschhorn syndrome candidate 1	[154]	[1]602952	nr	nr
Zeb1	Zinc finger E-box binding homeobox 1	[155]	[1]189909	Corneal dystrophy	nr
Zfp640/Mzf6d	Zinc finger protein 640	[87]	nr	nr	nr
Zic3	Zinc finger protein of the cerebellum 3	[156]	[1]300265	Congenital heart defects, nonsyndromic Heterotaxy, visceral, 1 VACTERL association	r
Cytoplasmic proteins					
Akap8/Akap95	A kinase (PRKA) anchor protein 8	[157]	[1]604692	nr	nr
Apaf1	Apoptotic peptidase activating factor 1	[158]	[1]602233	nr	nr
B9d1	B9 protein domain 1	[159]	[1]614144	Meckel syndrome 9	nr
Cask	Calcium/calmodulin-dependent serine protein kinase	[160]	[1]300172	FG syndrome 4 Mental retardation and microcephaly with pontine and cerebellar hypoplasia	r
Cdkn1c/p57kip2	Cyclin-dependent kinase inhibitor 1C	[161,162]	[1]600856	Beckwith-Wiedemann syndrome IMAGe syndrome	r
Chuk/Ikk1/Tcf16	Conserved helix-loop-helix ubiquitous kinase	[163]	[1]600664	Cocoon syndrome	nr
Crk	v-crk sarcoma virus CT10 oncogene homolog	[164]	[1]164762	nr	nr
Ctnnb1 (*KRT14-Cre*-mediated ablation)	Catenin (cadherin-associated protein), beta 1,	[165,166]	[1]116806	Mental retardation, autosomal dominant 19	nr
Cyp26B1	Cytochrome P450, family 26, subfamily b, polypeptide 1	[167]	[1]605207	Craniosynostosis with radiohumeral fusions and other skeletal and craniofacial anomalies	nr
Cyp51	Cytochrome P450, family 51	[168]	[1]601637	nr	nr
Dhcr7	7-dehydrocholesterol reductase	[169,170]	[1]602858	Smith-Lemli-Opitz syndrome	r
Dhrs3	Dehydrogenase/reductase (SDR family) member 3	[171,172]	[1]612830	nr	nr
Dicer1 (*Pax2-Cre*-mediated ablation)	Dicer 1, ribonuclease type Ⅲ	[29]	[1]606241	Rhabdomyosarcoma, embryonal, 2 Goiter, multinodular 1 Pleuropulmonary blastoma	nr
Dlg1/Dlgh/Sap97	Discs large 1	[173]	[1]601014	nr	nr
Fuz	Fuzzy planar cell polarity protein	[174]	[1]610622	Neural tube defects	nr
Gab1	Growth factor receptor bound protein 2-associated protein 1	[175]	[1]604439	nr	nr

Gad1/Gad67	Glutamate decarboxylase 1	[176,177]	[1]605363	Cerebral palsy, spastic quadriplegic, 1	r
Glce	Glucuronyl C5-epimerase	[178]	[1]612134	nr	nr
Glg1	Golgi apparatus protein 1	[179]	[1]600753	nr	nr
Grb2	Growth factor receptor bound protein 2	[180]	[1]108355	nr	nr
Gsk3b	Glycogen synthase kinase 3 beta	[181]	[1]605004	nr	nr
Hs2st1	Heparan sulfate 2-O-sulfotransferase 1	[182]	[1]604844	nr	nr
Hspb11/Ift25	Heat shock protein family B (small), member 11	[183]	[1]604844	nr	nr
Ilk (*Col2a1-Cre*-mediated ablation)	Integrin linked kinase	[184]	[1]602366	nr	nr
Impad1/Jaws	Inositol monophosphatase domain containing 1	[185]	[1]614010	Chondrodysplasia with joint dislocations, GRAPP type	r
Inpp5e	Inositol polyphosphate-5-phosphatase E	[186]	[1]613037	Joubert syndrome 1 Mental retardation, truncal obesity, retinal dystrophy, and micropenis	nr
Kif3a (*Wnt1-Cre*-mediated ablation)	Kinesin family member 3A	[187]	[1]604683	nr	nr
Map3k7/Tak1 (*Wnt1-Cre*-mediated ablation)	Mitogen-activated protein kinase kinase kinase 7	[188,189]	[1]602614	nr	nr
Nprl3	Nitrogen permease regulator-like 3	[190]	[1]600928	nr	nr
Ofd1 (*CAG-Cre*-mediated ablation)	Oral-facial-digital syndrome 1 gene homolog (human)	[191]	[1]300170	Joubert syndrome 10 Orofaciodigital syndrome Ⅰ Simpson-Golabi-Behmel syndrome, type 2	r
Pdss2 (*Pax2-Cre*-mediated ablation)	Prenyl (solanesyl) diphosphate synthase, subunit 2	[192]	[1]610564	Coenzyme Q10 deficiency, primary, 3	nr
Piga (*EIIa-Cre*-mediated ablation)	Phosphatidylinositol glycan anchor biosynthesis, class A	[193]	[1]311770	Multiple congenital anomalies-hypotonia-seizures syndrome 2 Paroxysmal nocturnal hemoglobinuria, somatic	nr
Pkdcc/Vlk (*Sox2-Cre*-mediated ablation)	Protein kinase domain containing, cytoplasmic	[194,195]	[1]614150	nr	nr
Prickle1	Prickle homolog 1	[196]	[1]608500	Epilepsy, progressive myoclonic 1B	nr
Rad23b	RAD23b homolog (*S. cerevisiae*)	[197]	[1]600062	nr	nr
Rspo2	R-spondin 2 homolog (Xenopus laevis)	[198,199]	[1]610575	nr	nr
Schip1	Schwannomin interacting protein 1	[87]	nr	nr	nr
Sdccag8	Serologically defined colon cancer antigen 8	[200]	[1]613524	Bardet-Biedl syndrome 16 Senior-Loken syndrome 7	nr
Slc32a1/Viaat	Solute carrier family 32, member 1	[201,202]	nr	nr	nr
Spry1 (*Wnt1-Cre*-mediated ablation)	Sprouty homolog 1	[203]	[1]602465	nr	nr
Spry2	Sprouty homolog 2	[204]	[1]602466	nr	nr
Sumo1 (heterozygous)	SMT3 suppressor of mif two 3 homolog 1 (yeast)	[205]	[1]601912	Orofacial cleft 10	r
Ugdh (*Wnt1-Cre*-mediated ablation)	UDP-glucose dehydrogenase	[206]	[1]603370	nr	nr
Wdpcp	WD repeat containing planar cell polarity effector	[207]	[1]613580	uc	nr
Extracellular proteins					
Col2a1	Collagen, type Ⅱ, alpha 1	[208]	[2]120140	Achondrogenesis, type Ⅱ Stickler syndrome, type Ⅰ Kniest dysplasia	r
Hspg2	Heparan sulfate proteoglycan 2, perlecan	[209,210]	[1]142461	Dyssegmental dysplasia Schwartz-Jampel syndrome, type 1	nr
Serpinh1/Hsp47 (*Col2a1-Cre*-mediated ablation)	Serpine peptidase inhibitor, clade H, member 1	[211]	[1]600943	Osteogenesis imperfecta, type X	nr
Smoc1	SPARC related modular calcium binding 1	[212]	[1]608488	Microphthalmia with limb anomalies	r

[1]Before an OMIM entry number indicates a gene; [2]Before an OMIM entry number indicates that the entry includes a description of a gene of known sequence and a phenotype. OMIM: Online Mendelian Inheritance in Man (http://omim.org); CL/P: Cleft lip and/or palate; r: Reported; nr: Not reported; uc: Unclarified.

beta receptor Ⅱ) in the neural crest or *Shh* (sonic hedgehog) in the epithelium result in failure of the palatal shelf development[10-13]. The fifth category is the persistence of medial edge epithelial (MEE) cells. The palatal epithelia are regionally divided into three parts: oral, nasal, and MEE. The MEE cells are removed from

Table 2 Classification of genes associated with cleft palate in mice

	Genes
Signaling pathway	
TGF-beta signaling pathway	*Acvr1/Alk2, Acvr2a, Bmp4*[1], *Bmp7, Bmpr1a/Alk3, Cdc42, Chrd, Crebbp/Cbp, Cited2, Foxc2/Mfh1*[1], *Foxd3, Foxe1/Titf2/Fkhl15*[1], *Foxf2, Fst, Inhba, Gdf11/Bmp11, Map3k7/Tak1, Pitx2, Smad4, Smad7, Tgfb2*[1], *Tgfb3*[1], *Tgfbr1/Alk5*[1], *Tgfbr2*[1]
Hedgehog signaling pathway	*Bmp4*[1], *Bmp7, Crebbp/Cbp, Gli2*[1], *Gli3*[1], *Gsk3b, Ptch1/Ptc1*[1], *Shh*[1], *Smo/Smoh, Wnt5a*[1], *Wnt9b*
Wnt signaling pathway	*Acvr1/Alk2, Ctnnb1, Crebbp/Cbp, Edn1*[1], *Fzd2, Gsk3b, Lrp6, Map3k7/Tak1, Prickle1, Smad4, Smo/Smoh, Wnt5a*[1], *Wnt9b*
FGF signaling pathway	*Fgf10, Fgf18, Fgf9, Fgfr1*[1], *Fgfr2*[1], *Grb2, Spry1, Spry2*
MAPK signaling pathway	*Cdc42, Chuk/Ikk1/Tcf16, Egfr, Fgf10, Fgf18, Fgf9, Fgfr1*[1], *Fgfr2*[1], *Grb2, Map3k7/Tak1, Pdgfra*[1], *Tgfb2*[1], *Tgfb3*[1], *Tgfbr1/Alk5*[1], *Tgfbr2*[1], *Crk, Itgb1*
Cytokine-cytokine receptor interaction	*Acvr1/Alk2, Acvr2a, Bmp7, Bmpr1a/Alk3, Egfr, Inhba, Pdgfra*[1], *Pdgfc*[1], *Tgfb2*[1], *Tgfb3*[1], *Tgfbr1/Alk5*[1], *Tgfbr2*[1], *Vegfa*
CBL mediated ligand-induced downregulation of EGF receptors	*Egfr, Grb2, Pdgfra*[1]
Sprouty regulation of tyrosine kinase signals	*Egfr, Grb2, Spry2, Spry1*
NFkB activation	*Crebbp/Cbp, Chuk/Ikk1/Tcf16, Map3k7/Tak1, Smad4, Tgfbr1/Alk5*[1], *Tgfbr2*[1]
Adherens junction	*Crebbp/Cbp, Ctnnb1, Cdc42, Egfr, Fgfr1*[1], *Map3k7/ Tak1, Smad4, Snai2, Tgfbr1/Alk5*[1], *Tgfbr2*[1]
Focal adhesion	*Ctnnb1, Cdc42, Col2a1*[1], *Crk, Egfr, Gsk3b, Grb2, Itga5, Itgb1, Itgb8, Ilk, Pdgfra*[1], *Pdgfc*[1], *Vegfa*
Steroid biosynthesis	*Cyp51, Dhcr7*[1], *Sc5d/Sc5dl*
Cell cycle	*Crebbp/Cbp, Cdkn1c/p57kip2*[1], *Gsk3b, Smad4, Tgfb2*[1], *Tgfb3*[1]
Regulation of actin cytoskeleton	*Cdc42, Crk, Egfr, Fgf9, Fgf10, Fgf18, Fgfr1*[1], *Fgfr2*[1], *Itga5, Itgb1, Itgb8, Pdgfra*[1], *Pdgfc*[1]
Axon guidance	*Cdc42, Efna5, Efnb1*[1], *Efnb2, Gsk3b, Itgb1*
Endocytosis	*Cdc42, Egfr, Fgfr2*[1], *Pdgfra*[1], *Tgfbr1/Alk5*[1], *Tgfbr2*[1]
Angiogenesis	*Ctnnb1, Crk, Efnb1*[1], *Efnb2, Fgfr1*[1], *Fgfr2*[1], *Fzd2, Gsk3b, Grb2, Jag1, Jag2, Pdgfra*[1], *Pdgfc*[1], *Vegfa, Wnt5a*[1]
Family	
Homeobox protein	*Alx1*[1], *Barx1, Alx3*[1], *Alx4*[1], *Dlx1, Dlx2, Dlx5*[1], *Gbx2, Gsc, Hoxa2*[1], *Msx1*[1], *Msx2*[1], *Pax9, Prrx1*[1], *Pitx1*[1], *Pitx2, Rax, Shox2, Vax1*[1]
Tgf-beta receptor type Ⅰ and Ⅱ	*Acvr1/Alk2, Acvr2a, Bmpr1a, Tgfbr1/Alk5*[1], *Tgfbr2*[1]
Tgf-beta family	*Bmp4*[1], *Bmp7, Gdf11, Inhba, Tgfb2*[1], *Tgfb3*[1]
Tyrosine protein kinase	*Egfr, Fgfr1*[1], *Fgfr2*[1], *Pdgfra*[1], *Ror2*[1], *Ryk*
Ephrin	*Efna5, Efnb1*[1], *Efnb2*
Zinc finger protein	*Gli2*[1], *Gli3*[1], *Zic3*[1], *Hic1, Snai2*
Forkhead protein	*Foxc2*[1], *Foxd3, Foxe1*[1], *Foxf2*
T-box protein	*Tbx1*[1], *Tbx2, Tbx22*[1]
Sox transcription factor	*Sox5, Sox9*[1], *Sox11*
Heparin-binding growth factor family member/FGF	*Fgf9, Fgf10, Fgf18*
Sprouty	*Spry1, Spry2*
Smad	*Smad4, Smad7*
Integrin beta subunit	*Itgb1, Itgb8*
Frizzled	*Fzd2, Smo*
Wnt related	*Wnt5a*[1], *Wnt9b*
Serine-threonine protein kinase	*Ilk, Mpa3k7/Tak1*
LIM domain containing protein	*Lhx8*[1], *Lhx7, Prickle1*
EGF-like domain protein	*Jag1, Jag2*

[1]Genes involved in human cleft lip and/or palate. TGF: Transforming growth factor; FGF: Fibroblast growth factor; MAPK: Mitogen-activated protein kinase; CBL: Casitas B-lineage lymphoma; EGF: Epidermal growth factor.

the fusion line by epithelial cell migration, apoptosis, and epithelial-mesenchymal transdifferentiation[14]. *Tgfb3* (transforming growth factor, beta 3) or *Egfr* (epidermal growth factor receptor) knockout mice lack the adhesive interactions between the palatal shelves because the fate of MEE cells is altered[15-18]. In the last category, the cleft palate arises as a secondary defect, due to tongue or bone anomalies during development. For example, *Hoxa2* (homeobox A2) knockout mice exhibit CP, because depression of the tongue is inhibited by the abnormal attachment of the hyoglossus muscle to the greater horn of the hyoid[19,20].

There is molecular heterogeneity along the medial-lateral and anterior-posterior axes of palatal shelves. Regionally restricted expression of molecules provides distinct regulatory mechanisms for the development of palatal shelves. For instance, *Msx1, Shox2* (short stature homeobox 2), *Fgf10* (fibroblast growth factor 10), *Bmp2* (bone morphogenetic protein 2), and *Bmp4* (bone

Table 3 Six categories of defects that result in cleft palate in mutant mice

	Defects	Knockout mice
(1)	Failure of the palatal shelf formation (small palatal shelves)	*Acvr2a*[34,50], [1]*Fgfr2*[13], [1]*Lhx8*[11], *Pitx2*[126], *Itga5*[65], *Fst*[46]
(2)	Abnormal fusion of palatal shelves and tongue or the mandible	*Jag2*[70], [1]*Irf6*[109,110], [1]*Tbx1*[4], *Fgf10*[41]
(3)	Failure or delayed palatal shelf elevation	*Pax9*[6], [1]*Pitx1*[7], [1]*Osr2*[9], [1]*Gli2*[8], [1]*Tgfb2*[55], [1]*Pdgfc*[51], *Dhrs3*[172]
(4)	Failure of the palatal shelf development after the elevation	[1]*Msx1*[10], [1]*Lhx8*[11], [1]*Tgfbr2* (*Wnt1-Cre*-mediated ablation)[12]
(5)	Persistence of medial edge epithelial cells	*Apaf1*[158], [1]*Tgfb3*[18], *Egfr*[17], *Ctnnb1* (*K14-Cre*-mediated ablation)[166]
(6)	Secondary defect	[1]*Hoxa2*[19,20], [1]*Satb2*[135], *Acvr1/Alk2* (*Wnt1-Cre*-mediated ablation)[33]

[1]Genes involved in human cleft lip and/or palate.

morphogenetic protein 4) are exclusively expressed in the anterior region of the palatal shelves[4,13,21,22]. The ablation of *Msx1* in mice results in cell proliferation alterations in the anterior palatal mesenchyme and cleft palate[23]. *Shox2* shows restricted expression patterns in the anterior palatal mesenchyme and the ablation of *Shox2* in mice results in anterior cleft palates[22]. *Fgf10* is also expressed in the anterior palatal mesenchymal cells and induces *Shh* expression through its receptor Fgfr2 (fibroblast growth factor receptor 2) in the palatal epithelium[13]. On the other hand, *Pax9* is expressed in the posterior palatal shelves. Ablation of *Pax9* results in cleft palates because of a palatal shelf development defect[6,21]. Even though it is known that Tbx1 induces the expression of *Pax9* in the posterior part of palatal shelves[4], the mechanism of Tbx1-induced *Pax9* expression during palatogenesis remains unknown. There is also molecular heterogeneity along the medial-lateral axis of the palatal shelf. For instance, *Osr2* expression in the palatal shelf is characterized by a medial-lateral gradient. Loss of *Osr2* results in the failure of palatal shelf elevation because of the delayed development of the medial part of palatal shelf[9].

MOLECULAR PATHOGENESIS OF CLEFT PALATES

Since most of the studies in mice focus on complete CP, the pathogenesis of other CP phenotypes is not well understood. *Tbx1* is expressed in the developing palatal shelves in mice[4], highlighting the crucial function of *Tbx1* in regulating palatal development. Loss of *Tbx1* results in the abnormal fusion of the oral epithelia, which induces CP by preventing the elevation of palatal shelves[4]. The phenotypic variation in the *Tbx1*$^{-/-}$ palates strongly suggests that Tbx1 is involved in modifier genes and/or stochastic factors. *Tgfb3*$^{-/-}$ mice also exhibit either incomplete or complete CP[15,16]. Ablation of *Shox2* results in anterior cleft palates[22]. Knockout mice of *Sall3* (spalt-like transcription factor 3), which is expressed in the palatal mesenchyme, show hypoplasia of the soft palate and epiglottis[24]. These mice are unique models for studying the etiopathogenesis underlying the variety of CP phenotypes in humans.

A comprehensive list of molecules associated with CL/P in mice and their classification should provide insights into the genetic etiology of CL/P; however, the phenotype of knockout mice does not always recapitulate the phenotype in humans (Table 1). Since Table 1 includes the genes associated with tissue-specific conditional knockout mice, mutations of these genes may induce the phenotype of embryonic lethality in humans. Haploinsufficiency mutations of the *TBX1* mutation are associated with CP[25]; however, heterozygous mice with *Tbx1* are phenotypically normal, and *Tbx1*$^{-/-}$ mice have CP phenotypes[4], thereby suggesting a species-specific requirement for *Tbx1* dosage. Mutations of the *PVRL1* (poliovirus receptor-related 1 or Nectin 1) cause CL/P-ectodermal dysplasia syndrome and nonsyndromic CL/P (OMIM #225060), whereas *Pvrl1*$^{-/-}$ mice do not develop CP[26]. Lack of palatal phenotypes in mice may be a consequence of functional redundancy of *Pvrl* genes. Interestingly, *Smad4*, *Smad7*, *Fgf9*, *Fgf10*, and *Fgf18* are involved in CP in mice (Table 1), whereas *SMAD3* (OMIM *613795), *FGF8* (OMIM *600483), and *FGF17* (OMIM *603725) are involved in CP in humans.

Candidate genes for nonsyndromic CP in human must show a relevant spatio-temporal gene expression pattern in the developing palatal shelves, and induce a specific cleft palate phenotype when deleted[1]. Disease genes responsible for Mendelian forms of syndromic CP are also important in the etiology of nonsyndromic CP[27]. *TBX1* mutations have been found in patients with incomplete CP without clinical diagnosis of del22q11.2 syndrome[25]. *TBX1* is also one of the disease genes of conotruncal anomaly face syndrome (OMIM #217095), which is often associated with cleft palates, particularly submucosal CP, and bifid uvula. *Tbx1*$^{-/-}$ palatal phenotype in mice makes *Tbx1* a potential candidate gene for nonsyndromic CP, especially submucosal CP and incomplete CP in humans.

RECENT ADVANCES IN PALATOGENESIS

Even though many genes associated with CP have been identified, little is known about how the environment influences gene expression in palatogenesis, and palatal phenotype. Epigenetics, such as DNA methylation and chromatin remodeling, and the microRNA (miRNA) regulation could change gene expression profiles and phenotypes. Hundreds of miRNAs, small non-

coding RNAs that modulate gene expression at the post-transcriptional level are expressed in murine embryonic craniofacial tissue[28]. Conditional knockout mice of *Dicer1* (dicer 1, ribonuclease type Ⅲ), which regulates the generation of miRNA, resulted in disrupted palatogenesis[29], suggesting that the miRNA function may be important in mammalian palatogenesis. miR-140, which modulates BMP signaling, regulates palatogenesis in mice[30] and miR-17-92 modulates Tbx1 and Tbx3 (T-box 3) activity, resulting in orofacial clefting[31]. Interestingly, transcription of *Dicer1* is regulated by TP63 (transformation related protein p63)[32], whose mutations are associated with cleft palate phenotypes (Table 1). Since genes involved in miRNA generation and individual CP genes can both be modulated by several miRNAs, it is conceivable that complex gene-miRNA interactions exist during palatogenesis. Genetically engineered mice with miRNAs, which modulate CP genes, may provide new information on the gene interactions underlying the palatogenesis. Further studies on miRNA and methylated genes involved in palatogenesis are necessary to understand the environmental factors contributing to CP.

CONCLUSION

Studies with genetically engineered mice with CP reveal the importance of regulated molecular functions in palatogenesis and provide the opportunity to discover new genes implicated in palatogenesis. However, there is still much to learn about transcriptional regulation and molecular networks in palatogenesis. The interactions between environmental/stochastic factors and genes in the etiopathogenesis of CL/P require further studies. Teratogenic effects of dioxins and retinoic acid have been reported in mice[1]. Mutant mice with CP can also be used as models to assess environmental effects or gene-environment interactions. Epithelial abnormal fusion could be one of the stochastic causes that induce a variety of CP phenotypes in mice. Understanding the palatal epithelial functions during palatogenesis may also lead to the discovery of novel therapeutic methods for CL/P.

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