Head and Neck Development and Malformations

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Learning Objectives

- Learn cranial neural crest cells and their contribution to craniofacial development

- Know the different components of the pharyngeal arch and their later fates, be able to recognize the pharyngeal origin of mature structures, and recognize which structures are non-pharyngeal. Developmental terms from this lecture that are of particular importance: pharyngeal arch, pouch, and cleft, frontonasal process.

- You should be able to recognize the developmental significance of innervation patterns and transitions in terms of the developmental origins of the innervated structures.

- Embryonic basis for cleft lip with or without cleft palate, cleft palate, and other craniofacial malformations.
Neural crest cells
Cranial neural crest cell migration
Rhombomeres and neural crest cell migration
Pharyngeal arches and their associated cranial nerves
Neural Crest Cell Fate Analysis using DiI injection
Labeling of the cranial nerves
Migration of cranial neural crest cells
Pharyngeal arches

In humans = pharyngeal arches
In fish = branchial arches

Ectoderm separating arches on outside = pharyngeal cleft (or groove)
Endoderm separating grooves on inside = pharyngeal pouch
The pharyngeal arches are fetal structures that constitute most of the nonneural elements of the head and neck. Humans (and other mammals) have **five pharyngeal arches (numbered 1, 2, 3, 4, and 6).**

A pharyngeal cleft (or groove) separates adjacent arches on the outside (ectoderm), and a pharyngeal pouch separates adjacent arches on the inside (endoderm).

In addition to ectoderm and endoderm, each arch has an artery, a nerve, a cartilagenous element, and mesenchymal cells.

The **first arch** has two subdivisions: the maxillary process and the mandibular process.

- The trigeminal nerve (the nerve of the 1st arch) has separate branches that innervate each (CNV2 and CNV3 respectively).

The **frontonasal process** (prominence) (FNP) in the anterior midline is similar to a pharyngeal arch (it has a nerve (CN V1) and mesenchyme and follows a similar developmental strategy) but is somewhat different as well (there is no pouch or cleft that demarks it, and no cartilage).
Pharyngeal arches

(note: 1st arch ectoderm (blue) extends to inside (of what will become the oral cavity))
Structures derived from pharyngeal arch components

<table>
<thead>
<tr>
<th>Arch</th>
<th>Nerve</th>
<th>Muscles</th>
<th>Skeletal Structures</th>
<th>Ligaments</th>
</tr>
</thead>
<tbody>
<tr>
<td>First (mandibular)</td>
<td>Trigeminal†(CN V)</td>
<td>Muscles of mastication†</td>
<td>Malleus</td>
<td>Anterior ligament of malleus</td>
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<tr>
<td></td>
<td></td>
<td>Mylohyoid and anterior belly of digastic</td>
<td>Incus</td>
<td>Sphenomandibular ligament</td>
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<tr>
<td></td>
<td></td>
<td>Tensor tympani</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tensor veli palatini</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second (hyoid)</td>
<td>Facial (CN VII)</td>
<td>Muscles of facial expression§</td>
<td>Stapes</td>
<td>Stylohyoid ligament</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stapedius</td>
<td>Styloid process</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stylohyoid</td>
<td>Lesser cornu of hyoid</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Posterior belly of digastic</td>
<td>Upper part of body of hyoid bone</td>
<td></td>
</tr>
<tr>
<td>Third</td>
<td>Glossopharyngeal (CN IX)</td>
<td>Stylopharyngeus</td>
<td>Greater cornu of hyoid</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower part of body of hyoid bone</td>
<td></td>
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<tr>
<td>Fourth and sixth††</td>
<td>Superior laryngeal branch of vagus (CN X)</td>
<td>Cricothyroid</td>
<td>Thyroid cartilage</td>
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<tr>
<td></td>
<td>Recurrent laryngeal branch of vagus (CN X)</td>
<td>Levator veli palatini</td>
<td>Cricoid cartilage</td>
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<tr>
<td></td>
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<td>Constrictors of pharynx</td>
<td>Arytenoid cartilage</td>
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<tr>
<td></td>
<td></td>
<td>Intrinsic muscles of larynx</td>
<td>Corniculate cartilage</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Striated muscles of esophagus</td>
<td>Cuneiform cartilage</td>
<td></td>
</tr>
</tbody>
</table>

*The derivatives of the aortic arch arteries are described in Chapter 14.
† The ophthalmic division does not supply any pharyngeal arch components.
‡ Temporalis, masseter, medial and lateral pterygoids.
§ Buccinator, auricularis, frontalis, platysma, orbicularis oris and orbicularis oculi.
†† The fifth pharyngeal arch is often absent. When present, it is rudimentary and usually has no recognizable cartilage bar. The cartilaginous components of the fourth and sixth arches fuse to form the cartilages of the larynx.
4 of the 12 cranial nerves innervate the pharyngeal arches

**Pharyngeal arch nerves**

- First arch: Trigeminal (CN V)
- Second: Facial (CN VII)
- Third: Glossopharyngeal (CN IX)
- Fourth and sixth: Vagus (CN X)

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- Each pharyngeal nerve is a mixed nerve, with a motor component to pharyngeal muscle cells, and a sensory component to pharyngeal epithelium (ectoderm or endoderm).
Pharyngeal arch cartilage
Pharyngeal arch muscle

Posterior belly of digastric muscle
Recall the general formation of most body muscles: are derived from paraxial mesoderm -> somites -> myotome -> muscle cells.

- Muscle cells obtain innervation early, and maintain that innervation as they migrate.

- Craniofacial muscles are also derived from paraxial mesoderm, except that somites do not form per se in the head (there is an analogous domain of paraxial mesoderm that is called “somitomeres”, not a term to know). **Craniofacial muscles also maintain their early innervation pattern once it is established.** Note that some craniofacial muscles migrate within pharyngeal arches, other do not.

Pharyngeal muscles intermingle and migrate widely though the head and neck. However, the origin of each muscle can always be determined by its innervation pattern.

The origin and innervation of tongue muscles is described below.
Innervation of cranial muscles

<table>
<thead>
<tr>
<th>Innervation</th>
<th>Muscles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oculomotor (III)</td>
<td>Sup, med, inf. recti</td>
</tr>
<tr>
<td>Troclear (IV)</td>
<td>Sup. oblique</td>
</tr>
<tr>
<td>Trigeminal (V)</td>
<td>Muscles of mastication</td>
</tr>
<tr>
<td>Abducens (VI)</td>
<td>Lat. rectus</td>
</tr>
<tr>
<td>Facial (VII)</td>
<td>Facial (various)</td>
</tr>
<tr>
<td>Glossopharyngeal (IX)</td>
<td>Stylopharyngeus</td>
</tr>
<tr>
<td>Vagus (X)</td>
<td>Palate and Intrinsic laryngeal</td>
</tr>
<tr>
<td>Hypoglossal (XII)</td>
<td>Tongue muscles</td>
</tr>
</tbody>
</table>

(Pharyngeal nerves and muscles in red)
General sensory innervation of oral cavity

Special sensory innervation of tongue

- CN VII (trigeminal) to anterior 2/3 of tongue
- CN IX and CN X to posterior 1/3

Motor innervation of tongue

- CN XII (12) to all tongue muscles except for palatoglossus (CN X)
Craniofacial mesenchyme

a loosely organized, mainly mesodermal embryonic tissue that develops into connective and skeletal tissues, including blood and lymph.

NC gives rise to:
- dura of brain
- ganglia of cranial nerves

Mesoderm gives rise to:
- striated muscle
- endothelium

Both give rise to:
- cranial bones
- vascular sm. Muscle

Pharyngeal mesenchyme has two developmental origins: mesoderm and neural crest. There are some unique fates for each of these (pharyngeal mesoderm is the source of cranial vascular endothelium and striated muscles, neural crest is the source of the dura) and some common fates (cranial bones have dual origins).
Migration of cranial neural crest cells and facial development
Normal lip formation
Unilateral and bilateral cleft lip with cleft palate
First arch syndrome

- Defects of external ear
- Mandibular hypoplasia
- Cleft palate (variable)
The anatomy of palatogenesis

6 wks

12 wks
The oral cavity is delimited by the FNP, maxillary processes, and mandibular processes. The **mandibular** processes fuse in the midline, the two (bilateral) **maxillary** processes do not fuse at first and are separated from each other by the distal end of the FNP (the intermaxillary segment). Externally, the FNP is the origin of the philtrum of the upper lip.
Formation of palate
Cleft palate

A: Lip
   - Incisive papilla
   - Hard palate
   - Soft palate
   - Uvula

B: Cleft uvula

C: Nasal cavity
   - Nasal septum

D: Cleft palate

E: Site of incisive foramen

F: Secondary palate

G: Nasal septum

H: Nasal septum
Cleft palate
(histology)
Cleft lip with cleft palate
Tooth Development
Molecular Regulation of Tooth Morphogenesis

**DENTAL LAMINA**
- BMP
- FGF
- SHH
- WNT

**BUD**
- BMP
- FGF
- Msx2
- PDGF
- Lef1
- SHH
- TGF-β
- WNT

**CAP**
- BMP
- p21
- FGF
- Msx2
- PDGF
- Lef1
- SHH
- TGF-β
- WNT

**BELL**
- BMP
- p21
- FGF
- Msx2
- PDGF
- Lef1
- SHH
- TGF-β
- WNT

**TERMINAL DIFFERENTIATION**
- enamel
- dentin
- pulp
- cementum

**ERUPTION**
- enamel organ epithelium
- inner enamel epithelium
- outer enamel epithelium
- stellate reticulum

**ACTIVIN**
- Barx1
- Dlx1
- Dlx2
- Gli1
- Gli2
- Gli3
- Lhx6
- Lhx7
- Msx1
- Msx2
- Pax9

**BMP**
- Barx1
- Cbfa1
- Dlx1
- Dlx2
- Gli1
- Gli2
- Gli3
- Lef1
- Lhx6
- Lhx7
- Msx1
- Pax9

**FGF**
- Barx1
- Cbfa1
- Dlx1
- Dlx2
- Gli1
- Gli2
- Gli3
- Lef1
- Lhx6
- Lhx7
- Msx1
- Pax9

**WNT**
- Barx1
- Cbfa1
- Dlx1
- Dlx2
- Gli1
- Gli2
- Gli3
- Lef1
- Lhx6
- Lhx7
- Msx1
- Pax9

**ds** = dental sac
**eoe** = enamel organ epithelium
**iee** = inner enamel epithelium
**oeo** = outer enamel epithelium
**sr** = stellate reticulum
Formation of tongue (epithelium)

The location of the **oropharyngeal membrane** represents the transition from ectoderm to endoderm in the oral cavity. This transition lies within the oral cavity, anterior to the 1st pharyngeal pouch. This is also the location of a transition in sensory innervation of the epithelium of the oral cavity from CN V (ectoderm) to CN IX (endoderm).

The anterior 2/3 of the tongue forms from **tongue buds derived from the 1st arch (= ectodermal, CN V innervation)**. Inside the oral cavity, the 3rd arch displaces the 2nd arch to the outside, such that the posterior 1/3 of tongue is derived from the 3rd arch (endodermal, CN IX), with the very posterior end of tongue derived from the 4th arch (endodermal, CN X).
Germ Layer Derivatives

A. Pharyngeal Grooves and Arches
   - Cervical sinus
   - 1st, 2nd, 3rd, 4th
   - Esophagus

B. Thyroid diverticulum
   - Foramen cecum
   - Tongue
   - Tubotympanic recess
   - Palatine tonsil
   - Inferior parathyroid bud from 3rd pharyngeal pouch
   - Superior parathyroid bud from 4th pharyngeal pouch
   - Ultimopharyngeal body

C. Tympanic cavity and pharyngotympanic tube
   - Tympanic membrane
   - Auricle
   - Palatine tonsil
   - Tonsillar sinus
   - Foramen cecum
   - External acoustic meatus
   - Lymphoid tissue
   - Skin of neck
   - Superior parathyroid gland
   - Inferior parathyroid gland
   - Thyroid gland
   - Thymus
   - Former site of cervical sinus
   - Ultimopharyngeal body

- Thyroid gland
- Inferior parathyroid glands and thymus
- Superior parathyroid glands
- Ultimopharyngeal bodies
Lingual thyroid
Foramen cecum of tongue
Accessory thyroid tissue
Hyoid bone
Tract of thyroglossal duct
Cervical thyroid
Pyramidal lobe of thyroid gland
Normal position of thyroid gland

Foramen cecum of tongue
Pharyngotympanic tube and tympanic cavity (pouch I)
Tonsillar sinus and surface epithelium of palatine tonsil (pouch II)
Tongue
Tract of thyroglossal duct
Larynx
Ultimopharyngeal body (pouch IV)
Pouch IV
Parathyroid glands
Pouch III
Thymus (pouch III)
Thyroid gland
Cervical thyroglossal duct cyst

A

- Thyroglossal duct cyst
- Hyoid bone
- Thyroid cartilage
- Thyroid gland
- Foramen cecum of tongue
- Opening of thyroglossal duct sinus

B

- Lingual thyroglossal duct cyst
- Hyoid bone
- Cervical thyroglossal duct cyst
Imaging of a thyroglossal duct cyst

Epiglottis

Thyroglossal duct cyst

Thyroid cartilage
Pre-Natal Diagnosis

- **Ultrasound**
  - Level II USN
  - Operator dependent
  - Isolated cleft palate difficult

- **Amniocentesis**
  - Does not detect isolated CL/CP

- **Chorionic Villus Sampling**
  - Does not detect isolated CL/CP
Craniofacial Team at CHLA

- Audiologist
- Pediatric Dentist
- Pediatric Pulmonologist
- Pediatrician
- Speech Pathologist
- Orthodontist
- Social Worker
- Pediatric psychologist
- Plastic Surgeon
- Oral Surgeon
- Otolaryngologist
- Craniofacial Nurse
- Geneticist
- Ophthalmologist
Cleft Lip and Palate Patient

Cleft Lip Repair
(3 months old)

Cleft Palate Repair
(1 year old)

Pharyngoplasty (speech dependant)

Alveolar bone graft
(7 or 8 years old)

Jaw Surgery
(15 to 20 years old)

Cleft Lip/Nose Revision

Nasoalveolar Molding
(2 - 5 weeks begin)

Cleft Lip and Palate Patient

10-15 Team Visits

Cleft Lip Rhinoplasty
(15 to 20 years old)
The Story of ashley
Nasoalveolar Molding
Two Component Genetic System for Indelibly Marking Neural Crest Cells

Wnt1 expression

R26R-Cre mediated LacZ expression

Wnt1 promoter

R26R promoter

Cre

Neo/pA

β-GAL

loxp

loxp

E 9.5
Contribution of cranial neural crest cells during tooth development
Genetic intersections of mouse and human cleft palate study

Mouse cleft palate models

Human cleft palate

MGI data base

ALX4
CHD7
COL2A1
DHCR7
EFNB1
EYA1
FGFR2
FLNA
FOXE1
GLI3
IRF6
KCNJ2
MSX1
OFD1
RECQL4
RUNX2
SATB2
SIX3
SOX9
TBX1
TCOF1
TFAP2A
TGFBR2
TWIST1
WHSC1

125
25
82
Cleft palate in humans

Complete cleft of hard and soft palate

Partial cleft of soft palate

Animal models

Wnt1-Cre; Tgfbr2^{fl/fl}

K14-Cre; Tgfbr2^{fl/fl}
Palatogenesis in mice

E11  E12  E13

E14  E15  E16
E13.0 palate
E13.0 palate
E13.0 palate
E13.0 palate
E13.0 palate
E13.0 palate
200μm
1. Palatal fusion in vitro and cell fate analysis

2. It provides an opportunity for rescue experiment
**TGF-β signaling and palatogenesis**

- **TGF-β mutation and non-syndromic cleft palate**
  - Lidral et al., 1998 Am J. Hum Genet

- **TGF-β receptor mutation and cleft palate**
  - Loeys et al., 2005, Nature Genetics 37, 275-281
Cleft palate in humans

Complete cleft of hard and soft palate

Partial cleft of soft palate

Animal models

Wnt1-Cre; Tgfbr2<sup>fl/fl</sup>

K14-Cre; Tgfbr2<sup>fl/fl</sup>
Major categories of cleft palate

TGF-β signaling in regulating the fate of
(1) medial edge epithelium and
(2) cranial neural crest-derived mesenchyme

Chai and Maxson, 2006,
To investigate the TGF-β signaling mechanism

Facts: (1) All *Wnt1-Cre;Tgfbr2* CKO mice develop complete cleft palate.
(2) TGF-β signaling is crucial for cranial neural crest cell proliferation and palatal shelf growth.

*The identification of TGF-β signaling downstream target genes is essential for the study of TGF-β signaling mechanism in regulating palatogenesis.*
Identification of up-regulated *Tgfb2* and *Tgfbr3* in *Tgfbr2*\(^{fl/fl}\);*Wnt1-Cre* Palate by microarray analyses

E14.5

Up-regulated: 66 genes

Down-regulated: 75 genes

Differential expression of *Tgfb* and *Tgfbr* family members

<table>
<thead>
<tr>
<th>AFFY_ID</th>
<th>Symbol</th>
<th><em>Tgfbr2</em>(^{fl/fl});<em>Wnt1</em> / Control</th>
<th>FDR</th>
</tr>
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<tbody>
<tr>
<td>1438303_at</td>
<td>Tgfb2</td>
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<td>1433795_at</td>
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<td>0.02718*</td>
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Ingenuity Pathway Analysis (IPA) software analysis
**Marfan's syndrome type I**
--Mutation in *FBN1*

**Marfan's syndrome type II**

**Loeys-Dietz syndrome**

Craniofacial malformations, skeletal defects and vascular problems (aneurysms and dissection)
--Mutation gene: *TGFBR1* and/or *TGFBR2*


--**Loeys-Dietz syndrome type I** *(Elevated TGF-β signaling)*

With craniofacial features including craniosynostosis, hypertelorism and *cleft palate* and/or bifid uvula

--**Loeys-Dietz syndrome type II**

Without craniofacial features
Modulation of TGF-β signaling rescues cleft palate

Fig. 10