Interactions of the endocrine system, bone and oral health
All bones are not equal!

Dense high proportion of cortical bone

High proportion of trabecular bone
Mandible

Functions: mastication, respiration, speech
Formation and Development of the Mandible
Bones are formed through two distinct processes:

**Intramembranous Ossification**

**Endochondral Ossification**
Endochondral Bone Formation (Endochondral Ossification)

Mouse embryo, E14.5

First, cartilage primordia are formed (blue). Then, osteoblasts replace cartilage (red).

Mouse embryo, E18.0
**Meckel’s cartilage:**

- 1st pharyngeal arch derivative
- Close positional relationship to mandible
- Does not become the mandible
- Malleus
- Incus
- Sphenomandibular ligament

**Formation of Mandible:**

- Dependent on Meckel’s cartilage
- Does not derive from Meckel’s cartilage
- Derives from mesenchymal condensations which form along the lateral aspects of Meckel’s cartilage (week 6)
- Intramembranous ossification (week 7) spreads rapidly along the lateral aspect of Meckel’s cartilage both anteriorly and posteriorly
Meckel’s Cartilage & the Mandible:

• Mandibular branch of trigeminal nerve develops together with Meckel’s cartilage

• Ramus of the mandible develops by ossification posteriorly into the 1st arch, turning away from Meckel’s cartilage

• Points of divergence marked by the lingula
Mandibular Development:

• Subsequent mandibular growth until birth is influenced strongly by appearance of three secondary cartilages (coronoid, condylar and symphyseal) and the development of muscular attachments.

• Secondary cartilages undergo endochondral ossification.

• Condylar cartilage is most significant contributor to mandibular growth.
Mandibular Formation

Forms by what type of ossification process ??
Mandibular formation occurs by both endochondral and intramembranous ossification.

Intramembranous: body, ramus

Endochondral: condyle, coronoid, symphysis
Directions of Mandibular Growth

Predominant growth vectors are posterior and superior

Grows directly toward its articular contact in glenoid fossa

(also a good example of “displacement”)
What happens if the mandible does not grow adequately?
Infant with evident *micrognathia* due to Pierre Robin Sequence

- Micrognathia (small mandible) = primary defect
- Glossoptosis (posterior displacement or retraction of the tongue)
- Cleft palate (lack of fusion of palatal shelves)
- Upper airway obstruction
- Etiology unknown

Bones are formed through two distinct processes:

**Intramembranous Ossification**

- Mesenchymal condensation
- Osteogenic front
- Suture
- Osteoblast
- Osteocyte

**Endochondral Ossification**

- Mesenchymal condensation
- Perichondrium
- Chondrocyte
- Hypertrophic chondrocyte
- Proliferating chondrocyte
- Periosteum
- Bone trabecule
- Bone collar

*Image source: TRENDS in Genetics*
Endochondral Bone Formation
(Endochondral Ossification)

Mouse embryo, E14.5

First, cartilage primordia are formed (blue). Then, osteoblasts replace cartilage (red).

Mouse embryo, E18.0
Sizes and shapes of each bone in skull / face determine the “shape.”

(Sadler, 2012)  (Mishina, 2014)

Human  Mouse
Cranial Base Synchondroses

SYNCHONDROSES OF THE CRANIAL BASE
Endochondral Bone Formation
(Endochondral Ossification)

Mouse embryo, E14.5

Mouse embryo, E18.0

First, cartilage primordia are formed (blue). Then, osteoblasts replace cartilage (red).
Sutural Bone Growth

• Bone deposition along osteogenic front of growing facial or cranial bone

• Suture contains precursor cells that differentiate to form osteoblasts along the edge of the growing cranial bone

• Some sutures normally fuse and some remain patent
Craniofacial Suture Function

1. Bone growth

2. Provide firm union between bony segments

3. Allow for mechanical movement between bony segments for response to mechanical stress
• Midfacial growth occurs by bone deposition at bony sutures and by periosteal apposition (bone deposition) and bone remodeling

• Facial growth requires freedom of movement of facial sutures
Disorders of the skull vault in human

Craniosynostosis caused by premature fusion of sutures

Wilkie AO & Morriss-Kay GM, Nature reviews, Genetics, 2001
Sizes and shapes of each bone in skull / face determine the “shape.”

**Intramembranous Bone Formation**

- mesenchymal condensation (proliferation)
- osteoprogenitor (proliferation & differentiation)
- osteoblast (osteogenesis)
- osteocyte (osteogenic maturation)
- apoptosis
Craniosynostosis?

Mutations in critical genes (FGF etc.)

Abnormal ossification: Suture fused

- Facial deformity
- Delays of brain growth
Craniosynostosis

- Clinical condition of premature cranial suture fusion
- Results in abnormal craniofacial shape, increased intracranial pressure
- Team treatment commonly includes surgery, genetic counseling, dentistry, orthodontics, medical and social support.
- High incidence (1/2500 live births)
- Occurs sporadically or as part of a genetic syndrome
- Severity depends upon timing and number of fused sutures
- Etiology: genetic, environmental, multifactorial
Clinical symptoms of Craniosynostosis are viable.

Sagittal synostosis/ scaphocephaly

Metopic synostosis/ trigonocephaly

Coronal synostosis/ lambdoid synostosis (Plagiocephaly)
## Causative genes for Craniosynostosis

<table>
<thead>
<tr>
<th>Gene</th>
<th>mutation</th>
<th>suture</th>
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<tbody>
<tr>
<td>FGF receptors</td>
<td>GOF</td>
<td>Coronal suture</td>
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<td>Axin1 and Fgfr1</td>
<td>Compound LOF</td>
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<tr>
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<tr>
<td>ERF</td>
<td>LOF</td>
<td>All sutures</td>
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<tr>
<td>Bmpr1a</td>
<td>GOF</td>
<td>Metopic</td>
</tr>
</tbody>
</table>
Syndromic Craniosynostosis:

- Human malformation syndromes
- Abnormal development of the craniofacial skeleton
- Craniosynostosis
- Facial skeleton abnormalities
- Digit abnormalities
- Associated with genetic mutations
Genetic Basis of Syndromic Craniosynostosis:

Apert, Crouzon, Pfeiffer, Jackson-Weiss: FGFR’s
Boston-Type: Msx2
Saethre-Chotzen: Twist

FGFR’s (Fibroblast Growth Factor Receptor)

Msx2 (a transcription factor)

Twist (a transcription factor)

Autosomal Dominant
Full Penetrance
Variable Expression
Craniosynostosis Associated FGFR Mutations
FGFR Structure

Ig = immunoglobulin like domain

TM = transmembrane domain

TK = tyrosine kinase domain, allows for receptor signaling

FGF binding
Molecular Genetics of Apert Syndrome

Mutation:
- Activating mutation in FGFR2 gene
- Mutation is in area of FGF binding site
- Two mutations only

Molecular phenotype:
- Enhanced FGFR to ligand binding
- FGFR2 binding to wrong ligands
- Mutations can arise spontaneously
- Risk of mutation increases with paternal age

Molecular Genetics of Crouzon Syndrome

Mutation:
- Activating mutation in FGFR2 gene
- Mutation is in area of FGF binding site
- Two common mutations with numerous additional less common mutations.

Molecular phenotype:
- Ligand independent FGFR signaling
- Mutations can arise spontaneously
- Risk of mutation increases with paternal age
**FGFR2 Mutations**

![Diagram of FGFR2 protein with highlighted Apert and Crouzon common mutations](image)

- **Apert mutations**
- **Crouzon common mutations**
Apert Syndrome:

- Full penetrance, variable expression
- Prenatal Coronal Synostosis
- Increased intracranial pressure
- NL to diminished IQ
- Brain abnormalities (defects in septum pellucidum)
- Proptosis, hypertelorism
- Midface hypoplasia (facial sutures)
- Class III malocclusion
- Progressive cervical spine fusions
- Tracheal abnormalities
- Digit abnormalities: syndactyly
- Higher risk with higher paternal age
Crouzon Syndrome:

- Full penetrance, variable expression
- Coronal Synostosis, rare pansynostosis
- Increased intracranial pressure
- Brain abnormalities (defects in corpus callosum)
- NL intelligence
- Proptosis, hypertelorism
- Midface hypoplasia (facial sutures)
- Class III malocclusion
- Vertebral fusions
- Stylohyoid ligament calcification
- Tracheal abnormalities
- Digit abnormalities: None
- Higher risk with higher paternal age
FGF signals have distinct roles in the developing skull vault

Missense mutations of FGF receptors (Gain-of-function mutations) lead to craniosynostosis. (Approx. 30% of cases)

Enhancement of endogenous FGF signaling pathway extracellular signal-regulated kinases 1 and 2 (ERK1/2) is observed.

**FGFR Function:**

Cellular competence for responsiveness to FGF’s

Cell to cell signaling $\rightarrow$ tissue boundaries, induction of cell behavior changes
Craniosynostosis can be treated by inhibition of FGF signaling.

Gain-of-function mutation for FGFR2

U0126 is a chemical inhibitor for ERK1/2 (signaling molecules).

Coronal suture (arrows) are fused in the mutant. Inhibition of FGF signaling by U0126 rescues premature fusion of the suture.
How can we target growth factor signaling for therapeutic use?

Multiple ligands interact with multiple receptors.

They share common functions, but they also have unique functions.

Growth factor signaling interact with each other.

Tissues interact with each other.

Specificity!
FGFR splice variants

**FGFR2IIIb (yellow)**
- Expressed in epithelial lineages (dural cells)
- Specific for ligands that are expressed by mesenchymal lineage cells

**FGFR2IIIc (green)**
- Expressed in mesenchymal lineages (osteoblastic cells)
- Specific for ligands that are expressed by epithelial lineage cells
Prenatal Maxillary Bone Growth

• Lingual surface: bone deposition
• Nasal surface: initially bone deposition, then bone resorption → nasal sinus
• Infraorbital surface: bone deposition and remodeling → growing eyeball
• Exterior anterior surface: bone deposition → increase in arch length
• Exterior posterior surface: bone deposition → increase in arch length
• Increase in arch length allows for development and enlargement of tooth buds
• Zygomatic (malar) secondary cartilage in developing zygomatic process also contributes to maxillary growth