Jaw Bone Development

2. Morphogenic movements of facial processes to form the upper and lower jaw

Medial nasal: Primary palate
Maxillary: Secondary palate

Begins at Week 7
Histological View of Palate Fusion

1. Palatal shelves vertically orientated before 7 weeks
2. Palatal shelves elevated
3. Palatal shelves fused by 9 weeks
Jaw Bone Development

3. Condensation and differentiation of CNCC

Intramembranous Ossification

- Mesenchyme → Osteoblasts → Osteocyte

- Matrix deposition → Calcium → Mineralization
Skull Vault Development
Skull Vault Development

1. CNCC from the forebrain populate the presumptive frontal bones through two waves of migration

1. Into the FNP
2. Into the cerebral hemispheres
2. Condensation and differentiation of mesenchyme

**Osteogenic Mesenchyme**

E12.5/7 weeks

E16.5/3.5 months

**Osteoblast Differentiation**

- Neural crest
- Mesoderm
- Alkaline phosphatase (early marker for bone)

**Skull Vault Development**
Skull Vault Development

3. Appositional growth of bone plates at the suture
Suture development:

Approximation of bones (b) to form the presumptive suture (ps)

Overlap of bones to form the suture (s).

Separation of bones by undifferentiated mesenchyme

Fusion of bones by remodeling suture matrix
Sutures regulate growth by organizing intramembranous ossification.

- CNCC derived
- Paraxial mesoderm derived
- frontal bone
- parietal bone
- skin
- dura
- mesenchyme
- coronal suture
- periosteum
- osteogenic front
- preosteoblasts
- osteoblasts
- osteocyte
- bone matrix
Craniofacial Malformations

- Morphological defects
  - Craniosynostosis
  - Frontonasal Dysplasia
  - Cleidocranial dysplasia
  - Treacher Collins
  - Orofacial Clefts

- Genetic causes for these malformations
Craniosynostosis

• Premature closure of one or more cranial sutures before brain growth is complete

• Abnormally shaped skull, impaired brain growth, mental retardation, seizures, and/or blindness

• Occurs 1/2500 live births.
Calvarial shape is characteristic for each type of sutural synostosis.

Sagittal synostosis

Coronal synostosis

Frontal synostosis

Kabbani and Raghuveer, 2004
Genetic epidemiology of craniosynostosis

- Sagittal: 55%
- Coronal: 25%
- Frontal: 15%
- Lambdoid: 5%

Unknown: 72%

- EFNA4: 2%
- FGFR3: 9%
- FGFR2: 9%
- Twist: 6%
- Chr: 2%

n=214

Wilkie and Morriss-Kay, 2005
TWIST

- Basic helix-loop-helix transcription factor expressed by CNCC

- Named after *Drosophila* mutant: a twisted larva caused by gastrulation defects and a lack of mesoderm

- **TWIST1** loss of function (LOF) mutations cause Saethre-Chotzen syndrome which includes craniosynostosis

  [Images of skulls showing differences between Wt and Twist1+/-]
FGFR’s

• Tyrosine Kinase receptors activated by the secreted ligand, Fibroblast Growth Factor

• Role in skeletogenesis of long bones and calvarial sutures

• Gain of function (GOF) mutation cause multiple disorders with craniosynostosis.

FGFR2 GOF (Apert syndrome)

FGFR1  FGFR2  FGFR3

Pfeiffer  Crouzon  Apert  Muenke

Wt  FgfR2 GOF

Wang et al., 2005
MSX2

• Homeodomain transcription factor expressed by CNCC
• Directly regulated by Bone Morphogenetic Proteins (BMP)
• Represses osteoblast differentiation
• Increase Msx2 function or dosage causes craniosynostosis

Ma et al., 1996

GOF MSX2

5p trisomy of MSX2

Shiihara et al., 2004

Overexpression of murine Msx2

Liu et al., 1995
Frontonasal dysplasia

- Median cleft face syndrome

- Variability: tip of the nose may be missing or nose may separate vertically into two parts

- Ossification defect in frontal bone
**Ephrin B1**

- LOF mutations in *Ephrin B1 (EFNB1)* causes X-linked Craniofrontonasal Dysplasia

- Females have frontonasal dysplasia, craniofacial asymmetry, craniosynostosis, and bifid nasal tip

- Males only mildly affected with hypertelorism: X-inactivation in females causes patches of activity.

Twigg et al., 2004
Ephrin

• Regulate cell/cell interactions during patterning (hindbrain segmentation and skeletal formation)

• Binding initiates bi-directional signaling cascade necessary for sorting

• Expressed in complementary patterns to Eph and interactions causes repulsion
Cleidocranial dysplasia

- Delayed ossification of frontal and parietal bones
- Dental anomalies include delayed loss of primary teeth, delayed eruption of permanent teeth, and supernumerary teeth
- Underdeveloped or missing clavicles
- Occurs 1/1,000,000 live births.
**RUNX2**

- LOF mutation in this Runt class transcription factor cause CCD

- Transcription regulator of osteoblast differentiation (bone matrix proteins) expressed in CNCC

- Essential for commitment of multipotent mesenchymal cells into osteoblastic lineage
Molecular pathways in skull vault development

- **Craniosynostosis and FND**
- **Boston Type Craniosynostosis**
- **Crouzon, and Muenke syndrome**
- **Ephrin**
- **Msx2**
- **FGFR3**
- **Twist1**
- **FGFR2**
- **FGFR1**
- **Runx2**

**Syndromes and Disorders**:
- Saethre-Chotzen syndrome
- Apert, Crouzon, and Pfeiffer syndrome
- Pfeiffer syndrome
- Cleidocranial dysplasia
Treacher Collins syndrome

- Single dominant mutation occurs 1/10,000 live births.
- Hypoplasia of the facial bones, cleft palate, and ear defects that result in conductive hearing loss.
- TCOF-1, POLR1C, POLR1D: regulators of ribosome biogenesis required for CNCC generation and proliferation.
Orofacial Clefts

- Most common birth defect, occurring in 1/750 births
- Multifactor: genes and environment
- Can occur as part of a syndrome or in isolation
Facial Cleft Locations

Clefts occur at the lines of union between converging facial processes

1. Unilateral or bilateral cleft lip
2. Unilateral facial cleft
3. Median cleft lip
4. Median mandibular cleft
Sonic Hedgehog (SHH)

- Secreted growth factor expressed in pharyngeal arch epithelium

- LOF mutations cause Holoprosencephaly

- Defects range from mild (only a single central incisor) to moderate (orofacial clefts) to severe (proboscis, cyclopia)
MSX1

- Homeodomain transcription factor expressed in CNCC closely related to MSX2

- Critical for epithelial-mesenchymal interactions during development

- LOF MSX1 results in nonsyndromic cleft lip, cleft palate, and tooth agenesis

Jumlongras et al., 2001
Proliferation and condensation of CNCC

CNCC push up into ectoderm to form dental papilla

CNCC differentiate into odontoblasts (pulp) and produce dentin

E-M interactions

Ectodermal thickening

Proliferation and condensation of CNCC

CNCC push up into ectoderm to form dental papilla

CNCC differentiate into odontoblasts (pulp) and produce dentin

Blue= NCC
Pink= ecto
What are the etiologies of craniofacial disorders?
What are the etiologies of craniofacial disorders?

The key to every biological problem must finally be sought in the cell.
-E.B. Wilson
What are the etiologies of craniofacial disorders?

Cranial Neural Crest Cell

Proliferation
Survival
Migration
Differentiation

Trainor Lab
CNCC growth in Treacher Collins Syndrome

Example: CNCC hypoplasia caused by decreased proliferation and increased apoptosis

Dixon J. et.al., 2006
CNCC Proliferation in Tooth Agenesis

Example: Decreased proliferation of CNCC in early cap stage leads to tooth agenesis in $Msx1$ knockout mice.

$Msx1$ expression

Cap stage, NCC (Blue)

Bell stage, NCC (Blue)

Han et al., 2003
CNCC Differentiation in Cleidocranial Dysplasia

Differentiation into osteoblasts

Example: Absence of osteoblasts in Runx2 knockout mice

Hoshi et al., 1999
CNCC Migration in Craniosynostosis

Example: Cell mixing at the suture boundary in craniosynostosis

Merrill et al., 2006
The cellular and molecular mechanisms that control craniofacial development are key to understanding the etiology of craniofacial disorders.