# Examination of Bone Ossification Markers in Cochlear Development

Jolie Chang, MD; Kristin Butcher, BS; Omar Akil, PhD; Rich Schneider PhD; Lawrence Lustig, MD; Tamara Alliston PhD

Department of Otolaryngology - Head and Neck Surgery, University of California, San Francisco

Department of Orthopedic Surgery, University of California, San Francisco

## Background

The otic capsule is unique from other bone in the body. It has limited bone turnover and remodeling and has been described as the “hardest” bone in the body (Chang 2010). Several developmental bone defects are associated with hearing loss including osteogenesis imperfecta, fibrous dysplasia, craniofacial dysplasias and craniosynostoses. Elucidating otic capsule development will lead to a better understanding of the pathogenesis of these developmental bone diseases and how they affect hearing function. The goal of this study is to examine the spatial and temporal expression pattern of developmental ossification marker genes in the otic capsule.

Skeletal development during embryogenesis occurs through one of two mechanisms:

- **Intramembranous ossification**: embryonic mesenchyme cells directly differentiate into osteoblasts which produce mineralized bone (seen in mandible, skull, and clavicle).

- **Endochondral ossification**: mesenchyme develops through a cartilaginous intermediate stage followed by osteoblast development and ossification (seen in long bones, vertebrae, and otic capsule).

## Objective

To examine and describe the spatial and temporal expression pattern of developmental bone markers in the otic capsule.

## Methods

**Mice**: FVB and C57/B16 background strains. Wildtype mice were harvested at ages E19, P5, P10, P12, and P21. Cochleae were fixed, decalcified, embedded, and cryosectioned.

**In Situ Hybridization**: cDNA radiolabeled probes were used and positive hybridization identified by collecting emissions from the radioactive element. Slides were counterstained with blue nuclear DAPI dye. Each timepoint and probe was repeated 2-4 times to ensure reliability of the results.

## Results

### Figure 1: The developing cochlea.

- **Figure 2**: Control and nerve markers. Slides are stained with blue DAPI nuclear stain and probe signal shows up as white. Sprouty2 is expressed in the developing organ of corti and is expressed by chondrocytes at this stage. FGF-3 is expressed in the developing otic capsule and the organ of corti matching prior studies of these probes (Shim 2005).

### Figure 3: Early chondrogenesis.

- **Figure 4**: Chondrocyte hypertrophy and angiogenesis. In the third stage of endochondral ossification, chondrocytes hypertrophy and secrete factors to induce angiogenesis (VEGF). MMP13 is a collagenase and a marker of chondrocyte hypertrophy. MMP13 is maximally expressed in the endochondral layer of the otic capsule at P5.

### Figure 5: Osteogenesis.

- **Figure 6**: Ossification occurs from inner osteoblast differentiation and cartilage matrix degradation. Runx2 is a transcription factor that regulates osteoblast differentiation and is expressed in hypertrophic chondrocytes and differentiating osteoblasts. Early on, Runx2 is active at the edges of the developing otic capsule then subsequently in the modiolar bone. Osteocalcin (OC) is expressed in mature osteoblasts and is expressed in the periosteum at P21.

### Figure 7: The otic capsule exhibits limited bone remodeling.

Ephrins have recently been implicated in bone remodeling as factors that couple osteoblast and osteoclast activity and control new bone deposition. They also play a role in neuronal development and angiogenesis in the cochlea. Both EphB4 and Ephrin A2 are active early in cochlear development, however there is limited expression in the otic capsule at P21 consistent with prior studies describing limited bone remodeling within the mature cochlear capsule.

## Conclusions

- The developing otic capsule exhibits progression of developmental bone marker gene expression consistent with the stages of endochondral ossification.

- Contrary to prior descriptions, the otic capsule osteocytes mature from the inner endosteal layer to the outer periosteal layer.

- As predicted, there is limited expression of bone remodeling markers in the mature otic capsule given the low turnover rate.

## References