Stoichiometry of actin capping protein *CAPZB* Regulates Palate and Mandibular Morphogenesis

Kusumika Mukherjee[1,4], Michael J. Grimaldi[1], Michael Talkowski[2,4], James Gusella[2,4], Cynthia C. Morton[3,4], Richard Maas[3,4], and Eric C. Liao[1,4]

[1]Center for Regenerative Medicine, Massachusetts General Hospital, [2]Center for Human Genetic Research, Massachusetts General Hospital, [3]Brigham and Women's Hospital, [4]Harvard Medical School, Boston, USA.

Introduction: Orofacial clefts are among the most common congenital anomalies. The genetic basis for cleft palate and other craniofacial anomalies is being elucidated. The Developmental Genome Anatomy project (DGAP) has developed whole genome sequencing strategies to identify genes contributing to such human congenital anomalies. The isolated disruption of *CAPZB* was identified in a 6-month old patient presenting with cleft palate, micrognathia and hypotonia. We exploit the zebrafish model to determine the function of *capzb* and to understand the role of actin dynamics in craniofacial development and cleft pathogenesis.

Methods: The Meckel's cartilage and ethmoid plate are analogous to the mammalian mandible and primary palate respectively, making zebrafish an ideal model to study the genetic and developmental basis of palate and lower jaw morphogenesis. The spatiotemporal gene expression of *capzb* is determined by whole mount *in situ* hybridization (WISH) during early embryogenesis. Furthermore, craniofacial cartilaginous structures and muscles are examined in the *capzb* mutant identified from an insertional mutagenesis screen.

Results: WISH analysis shows that *capzb* is ubiquitously expressed, demonstrating its potential requirement in the function of many tissue types. Preliminary analysis of the *capzb* mutant show that the lower jaw elements are smaller and retrusive and the palate is only partially fused, leading to a cleft. The actin cytoskeleton is disorganized without capzb, leading to loss of cell morphology in the palate chondrocytes. The *capzb* mutants also show highly disorganized myofibrils leading to atrophied muscles. Interestingly, over-expression of Capzb is also disruptive, leading to myofibril disorganization and embryonic lethality.

Conclusion: We successfully modeled the phenotypes observed in the DGAP patient, in the zebrafish. We show that the *capzb* mutants exhibit micrognathia, cleft palate and atrophied muscles. We identify *CAPZB* to be important in craniofacial and muscle morphogenesis, disruption of which is pathologic for both palate and muscle development. Preliminary results from characterization of the *capzb* mutant show that defects in cell migration is responsible for some of the phenotypes in the mutants. Our ongoing gain and loss of function studies suggest that the stoichiometry of CAPZB regulates cranial neural crest migratory behavior, which translates to alterations in craniofacial form: providing molecular basis of both form and function through the relative concentration of a single cytoskeletal regulator.