Knockdown of Hedgehog signaling pathway contributes to the midline craniofacial development and facial elongation in the lizard *Anolis sagrei*

Greta M. Keller | Marta Marchini | Naaz Khan | Rushabh Shah | Thomas J. Sanger

Department of Biology | Loyola University Chicago

**Abstract**

- Great variation exists in the skeletal composition of amniote face and skull (Figure 1).

- The developmental bases of this variation remains unknown. Historically, most developmental studies have focused on only two species, “the” chick and mouse.

- The goal of this project is to understand the role Hedgehog signaling plays in squamate facial development in the lizard *Anolis sagrei*. We hypothesized that Hedgehog signaling disproportionately regulates the development of the midline facial skeleton compared to the lateral maxillary derivatives.

- Our research highlights that modifications in early craniofacial patterning may lead to diversity in the amniote skull.

**Craniofacial Diversity**

![Phylogeny of the major amniote groups illustrating the differences in skeletal proportions creating the snout/face (red premaxilla, blue nasals, and orange maxilla). In each of these species, the face forms from a series of facial prominences, yet the size, number, and proportion of those prominences varies among species.](image)

**Step 1: RNA-FISH**

**Part 1:** Add all HCR probe sets

**Part 2:** Add all HCR amplifiers

We use Fluorescent In Situ Hybridization to describe the expression of Sonic Hedgehog (Shh) during facial patterning of *A. sagrei*.

**Step 2: Egg Soak**

We soaked *A. sagrei* in 100uM cyclopamine or a DMSO control at day 0 or 2 of development to knock down Hedgehog signaling during craniofacial patterning.

**Step 3: Phenotyping**

We examined embryonic phenotypes of experimental and control embryos just prior to hatching.

**Shh expression during *A. sagrei* facial patterning**

![Fluorescent images of *A. sagrei* embryo heads highlighting Shh expression (top row, orange) and embryonic morphology (bottom row). Expression of Shh is located throughout the migratory and post-migratory neural crest during the early stages of facial development before moving to the oral ectoderm in stage 5. Shh expression becomes more restricted as facial development progresses.](image)

**Conclusion**

1) Shh is expressed throughout early craniofacial patterning, but exhibits a dynamic pattern of expression.

2) Both midline and lateral skeletal elements are reduced in size following disruption of Hedgehog signaling.

**Future Directions**

1) Compare patterns of proliferation in the midline and lateral facial prominences in control and treated embryos.

![Fluorescent images of Edu treated *A. sagrei* embryos of day 1 of development in treated (left) and control (right). Cross section of the maxillary prominence boxed.](image)

2) Determine whether the shortened lateral process are due to disruption of facial patterning or secondary disruption of facial outgrowth.

**Acknowledgements**

Thank you to the Loyola University Chicago Department of Biology for the use of the micro-CT scanner and microscope. Thank you to Naaz, Rushabh, and GiHo for your hard work to make this project possible. Thank you to Dr. Marta Marchini and Dr. Thomas Sanger for their guidance and support.