Oct 12, 2018

Editor

JOVE

Dear Editor,

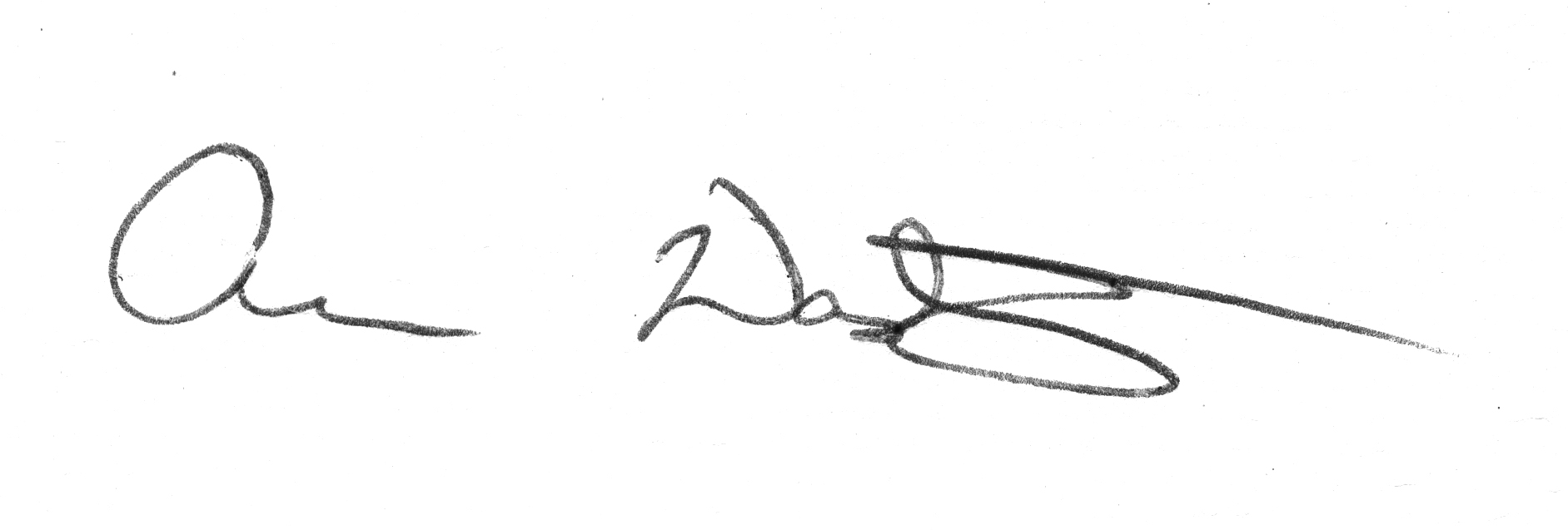
# I would appreciate your consideration of our manuscript (*Visualization of a novel superior ocular fissure during Danio rerio embryogenesis*),for publication as a Research Article in *JOVE*. Our research presents a methodological follow-up to our recently published work in *PLOS Genetics* (Morphogenetic defects underlie Superior Coloboma, a newly identified closure disorder of the dorsal eye, Hocking et al. 2018).

Our previous study began with the identification of eight patients with superior coloboma, a congenital ocular anomaly characterized by gaps in tissues of the superior iris, retina or lens. The similarity to coloboma (a disorder caused by failure to close the inferior ocular fissure), suggested the hypothesis that superior coloboma represented a new embryonic fissure disorder. Yet, existing models of vertebrate eye development describe a continuous dorsal optic cup. Following up 2 reports from more than 20 years ago, we revisted morphogenesis of the dorsal eye, and identified an anatomical structure within the dorsal zebrafish, chick, mouse and newt eye, *the superior fissure*. We provided evidence that failure to close this fissure results in superior coloboma.

This manuscript describes three separate techniques for examining molecular genetic regulation of superior fissure closure. We suggest that analyses of superior fissure closure delay occur at 28 hpf and that investigators primarily utilize either stereomicroscope observation (protocol 1) or Laminin immunohistochemistry (protocol 2). To complement such work and visualize the cellular dynamics of fissure closure we present a method for injecting zebrafish with membrane labeled GFP (protocol 3).

The main purpose for the existence of this manuscript is to standardize protocols such that examinations of superior fissure closure will take place using similar methodologies in multiple laboratories. As this field is just beginning and we have recently identified new patients, we think this will serve as an essential tool to an emerging research area.

Yours sincerely,



Dr. Andrew J. Waskiewicz Ph.D.

Professor, Department of Biological Sciences