

## Investigating the Mechanisms of Aniridic Cataract

Katie A. Coscia, Samuel G. Novo, Mahbubul H. Shihan, Yan Wang, Paige Faasumalie, Adam Pater-Faranda, and Melinda K. Duncan

Aniridia is a congenital eye disorder hallmarked by an absent or malformed iris and underdevelopment of the fovea and optic nerve. These structural deformities are associated with many complications like photophobia, decreased quality of vision, and an increased predisposition to the premature onset of vision-threatening diseases such as glaucoma, keratopathy, and cataracts. Patients with aniridia often require lifelong ophthalmological care, including many costly, invasive surgeries. Aniridia is caused by heterozygous inheritance of a mutation in the *PAX6* gene, a key regulatory transcription factor crucial for the proper formation and maintenance of the tissues of the eye. This haploinsufficiency of the *PAX6* gene is implicated in the eye's failure to maintain lens clarity and premature development of cataracts in aniridic patients. To explore the molecular mechanisms behind cataract development, several fibrotic markers with altered levels of transcription were identified via mRNA sequencing of wild type and *PAX6* mutant lens epithelial cells. Immunohistochemical staining was performed to investigate the role that four indicators of potential fibrosis play in the manifestation of the aniridic phenotype. There was a noticeable upregulation in the expression of  $\alpha$ -smooth muscle actin protein in the lens cells that correlated with the upregulation shown in the RNAseq data. In contrast, while fibronectin 1 and collagen I mRNA levels are also upregulated by RNAseq, no discernible changes in their protein expression levels were detected. Further, while the mRNA levels of extracellular matrix protein 1 are upregulated, the levels of this protein appear to be downregulated. Such discordant results suggest that the protein levels of ECM1, fibronectin 1 and Collagen I may be controlled via post-transcriptional mechanisms such as translational control. Overall, these results suggest that the *PAX6* heterozygous lens is sensitized to undergo fibrosis, which may explain the propensity of the aniridic lens to develop early-onset cataract.