Establishing a zebrafish model for *Crb2* mutation induced glomerular disease

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Chronic kidney disease (CKD) is characterized by the loss of kidney function over time. In the United States, CKD is prevalent in over 10% of the general population (Eckardt et al. 2013). As CKD progresses, it eventually progresses to end-stage renal disease, requiring a transplant or regular dialysis, due to complete kidney failure. Glomerular kidney diseases—those associated with glomerular filtration impairment—account for 90% of all end-stage renal disease cases (Wiggins 2007). Recently, mutations in the human Crumbs gene family, specifically those in the gene, *Crb2*, have been found to cause steroid-resistant nephrotic syndrome—a glomerular kidney disease—resulting in renal failure (Ebarasi et al. 2015).

To replicate the *Crb2* human disease phenotype in *Danio rerio* (zebrafish), we designed two translation-blocking morpholinos targeting the zebrafish *Crb2* ortholog, *crb2b*. Morpholinos are commonly used in biology to prevent the target gene from being expressed; this approach to genetic modification is referred to as *knockdown*. After the zebrafish embryos were injected with the morpholinos, the zebrafish were monitored throughout development for abnormal phenotypes and kidney injury. In these preliminary experiments, we aimed to determine whether the morpholinos knocked down of *crb2b* and whether the knockdown induced a nephrotic syndrome phenotype in zebrafish. Ultimately, the project goal is to establish a functional model of *Crb2* mutation induced glomerular disease in order to test potential drug compounds for disease treatment.

Unfortunately, the two morpholinos we designed to knockdown *crb2b* were not effective. Zebrafish injected with the morpholinos had severe developmental defects, including failure to develop heads and developing extremely small heads and eyes. While the morpholinos were causing kidney injury at high concentrations, overall zebrafish survival across experiments was highly inconsistent. Due to the difficulties establishing a *Crb2* mutation induced glomerular disease model with the translation-blocking morpholinos, future research will focus on different methods, in which knockdown can be induced later in development.

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