# Using CRISPR-Cas9 to knock out the Sterile Alpha Motif domain in the *Bicaudal C* gene of zebrafish (*Danio rerio*)

Veterinary Research Scholars Program
University of Missouri

Austin D. Kimes[1], Daniel J. Davis[2], Suzanne E. Ridenhour[1], Elizabeth C. Bryda[1,2]

[1]Department of Veterinary Pathobiology, College of Veterinary Medicine and [2]Animal Modeling Core, University of Missouri, Columbia, Missouri

## Abstract

Genetic alterations in the *Bicaudal C* (*Bicc1*) gene have been shown to cause renal cysts in humans, mice, and zebrafish. The Bicc1 protein has two types of functional domains: a Sterile Alpha Motif (SAM) and multiple tandem K Homology (KH) domains. The KH domains are important for RNA binding and the SAM domain is involved in protein-protein interactions. We have shown previously that morpholino knockdown of the zebrafish bicaudal c gene, *zbicc2*, causes renal cysts, however, point mutations expected to alter the SAM domain only, did not result in a discernible phenotype. These same point mutations do cause renal cysts in both humans and mice. Our hypothesis is that the function of the *zbicc2* SAM domain in the kidney has not been evolutionarily conserved. In order to test this, the region coding for the SAM domain of *zbicc2* was selectively knocked out using CRISPR-Cas9 genome editing in zebrafish embryos. Embryo development was monitored and gross morphology was observed until 5 days post-fertilization, a time point at which the fish kidney is fully developed and functional. DNA was extracted from the fish and will be analyzed by PCR to identify fish with a deletion of the region of *zbicc2* that codes for the SAM domain. Correlation between any genetic alterations and renal phenotype will be noted. The results of these studies will guide the appropriateness of future use of zebrafish as a model organism to study Bicc1 function in the kidney.

## Introduction

- Mutated *Bicaudal C* has been linked to renal cyst formation in humans, mice, and zebrafish.

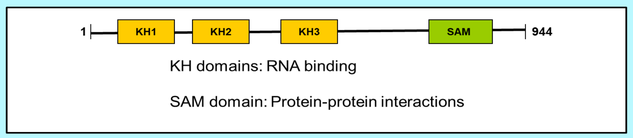


**Figure 1:** Graphical representation of the functional domains within the zebrafish version of the Bicaudal C protein (zbicc2).

- When *zbicc2* was knocked out in zebrafish (mutant zebrafish), the lack of *zbicc2* expression resulted in renal cyst formation (1).
- When mouse *Bicc1* RNA was added to mutant zebrafish embryos, the phenotype was rescued and there were no renal cysts (1).
- When human *BICC1* RNA was added to mutant zebrafish embryos, the phenotype was rescued and there were no renal cysts (1).
- When human *BICC1* RNA with point mutations predicted to cause cysts in human patients was added to mutant zebrafish embryos, the phenotype was not rescued and there were renal cysts (E.C.Bryda, unpublished data).

## Hypothesis

The function of the *Bicaudal C SAM* domain in the kidney has not been evolutionarily conserved between mammals (mouse/human) and zebrafish.

## Materials and Methods

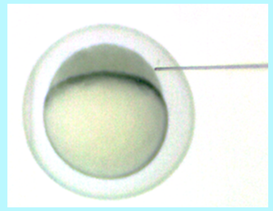

**Figure 2:** Delivery of CRSIPR-Cas9 reagents. The gRNAs and Cas9 protein were microinjected into zebrafish embryos. This image shows the microinjection procedure in a 1-cell embryo. The microinjection needle can be seen to the right of the embryo.

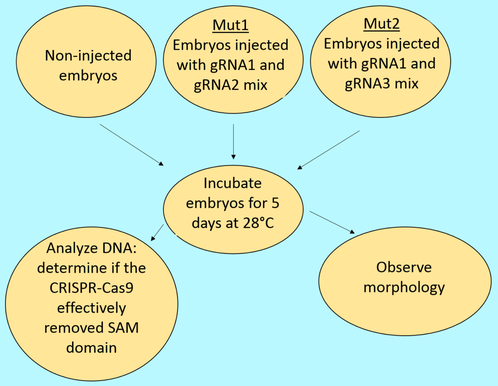


**Figure 3:** Experimental design. Two groups of embryos (Mut1 N=38 and Mut2 N=30) were injected with 75ng of each gRNA, 150µL Cas9 protein, and red dye at the 4- or 8-cell stage of development. One group (N=42) was not injected (control). Embryos were incubated at 28°C for 5 days. Kidneys are fully functional at this stage of development. The gross morphology of the embryos was recorded. Embryos were then euthanized, DNA was isolated and PCR analysis was performed to evaluate whether the SAM domain of *zbicc2* had been deleted.

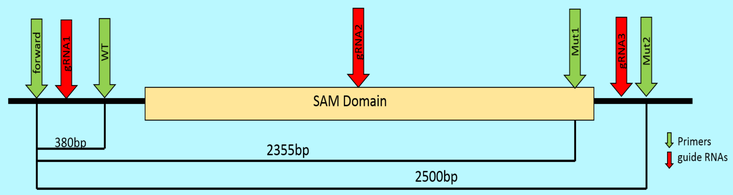


**Figure 4:** Diagram showing location of primers (green arrows) and gRNAs (red arrows) in relation to the SAM domain. The expected PCR amplicon size (in base pairs) for each primer set, when amplifying wild type (WT) DNA, is shown.

## Morphological Analysis

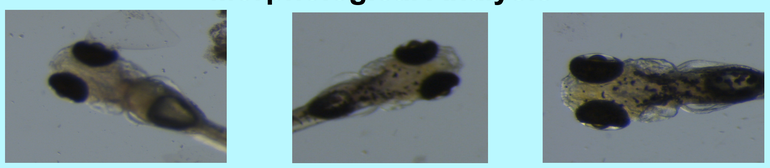

**Figure 5:** Embryo morphology. Ventral view of representative 5 day old larvae from each group. **Left:** non-injection group; **Middle:** Mut1 group (injection of gRNA1 and gRNA2); **Right:** Mut2 group: injection of gRNA1 and gRNA3. There were no morphological differences between the groups.

## PCR Analysis

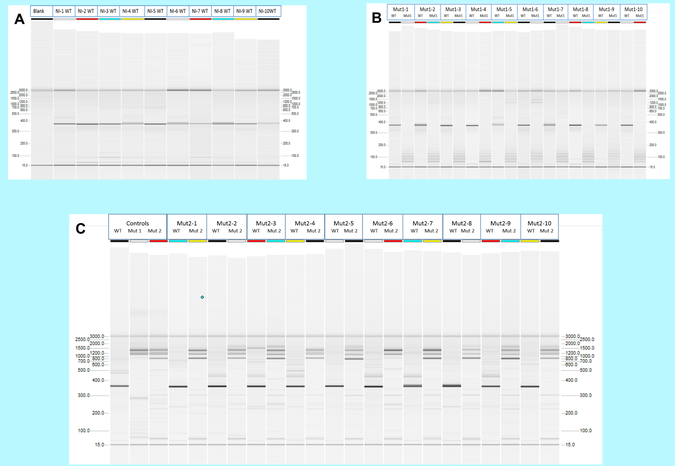


**Figure 6:** Images of the results of the PCR analysis for each group. **A)** Non-injected group (n= 10) using forward and WT primers. All fish were homozygous for the 380 bp WT amplicon. **B)** Mut1 group (n=10) using forward/WT primer set (odd number lanes) and forward/Mut1 primer set (even number lanes). All fish were homozygous for the 380 bp WT amplicon expected with the first primer set. No amplication was seen with the second primer set due to a technical failure of this primer set. **C)** Mut2 group (n=10 using forward/WT primer set (WT) and forward/Mut2 primer set (Mut 2). In the PCR reaction using the forward/Mut2 primer set, deletion of the SAM domain was expected to result in a 240 bp amplicon. Fish 3, 5, 6, 7, and 10 had this 240 bp amplicon. In all images, the molecular size standards (in bp) are shown on the left and the right of the image. PCR reactions were analyzed on a QIAxcel instrument. Alignment markers are present in each lane at 3 kb and 15 bp.

## Conclusions

- Successful deletion of the SAM domain of *zbicc2* was detected in some fish from the Mut2 group.
- There were no obvious gross morphological differences between the groups.
- Future studies will focus on histological analysis of kidneys from fish carrying deletion of the *zbicc2* SAM domain to look for the presence of renal cysts.
- Because there were no morphological differences and the SAM domain was knocked out, the SAM domain is not evlountionarily conserved across species.

## Acknowledgements

We would like to thank Dr. Anand Chandrasekhar and his lab for providing the embryos and Boehringer Ingelheim for student support.

## References

(1) Bouvrette, DJ *et al*. (2010). *Knockdown of bicaudal C in zebrafish (Danio rerio) causes cystic kidneys: A nonmammalian model of polycystic kidney disease.* Comp Med **60**:96-106.