Elucidating the Function of an Evolutionarily Conserved Embryonic Splice Variant of *Type II Collagen* During Vertebrate Development

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INTRODUCTION

During embryogenesis, complex coordinated development of structures are mediated by changes in spatiotemporal expression of many genes. One such genes is the *type II collagen alpha 1* (*col2a1*), which encodes a component of type II collagen fibrils that will directs the formation of cartilage of the embryonic skeleton. Much of this cartilage will undergoes ossification to become bone. While the major role of *col2a1* expression, during late development and into the mature organism, is as a vital component for skeleton formation, an embryonic specific splice variant has been suggest to play an important role in embryonic patterning.

The skeletal development of zebrafish follows a simple pattern that is highly conserved among all vertebrates. Although the last common ancestor of humans and zebrafish existed approximately 420 million years ago, both form the same skeletal cell types, including endochondral and dermal bones, as well as cartilages that persist into the adult. Interestingly, zebrafish contains two orthologues of the *col2a1* gene, *col2a1a* and *col2a1b*. Like in humans, *col2a1a* also expresses a similar embryonic splice variant during embryogenesis. Due to the advantages of visualization and analysis in zebrafish we are enabled in-depth study of early embryonic requirements of *col2a1* not possible in mammals due to access and size of mammalian embryos. This makes the zebrafish an excellent genetic model to study the function of the early embryonic *col2a1* splice variant during vertebrate trunk formation.

The goal of our study is to observe early development of our vertebrate model organism while manipulating the levels of the embryonic splice variant of *col2a1a*. Understanding the relationship between *col2a1a* and embryonic development will help elucidate the function of the *col2a1* splice variant during embryogenesis.

Structure of *col2a1a*

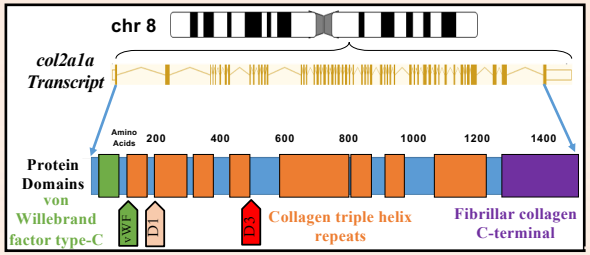

Figure 1: Schematic of the *col2a1a* gene transcript and its protein domains.

Expression of *col2a1a and BMP signaling*

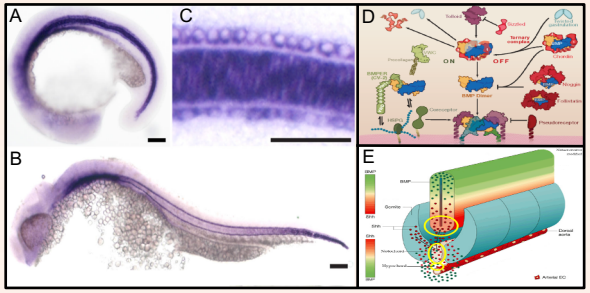

Figure 2: Expression of *col2a1a* during embryonic development.
Utilizing *in situ* hybridization analysis, our lab has demonstrated the pattern of expression of *col2a1a* at A) 15 somites, B) 24 hpf. *col2a1a* is also expressed in C) the notochord, floor plate, and hyperchord at 15 somites. D) *col2a1a* encodes for vWFC, which regulates BMP activation. E) BMP forms a concentration gradient with the Shh gradient to regulate dorsoventral patterning in the notochord.

RESULTS

Overexpression plasmids tend to not pass on to the next generation of transgenic zebrafish

Table 1: Transgenic overexpression of just the VWF-C domain

Plasmid Number	Crossing events for all fish	Stable Transgenic Zebrafish
R2:vWFC-dTomato	9	37

Knockdown of the embryonic *col2a1a* VWF-C causes developmental delay and defects

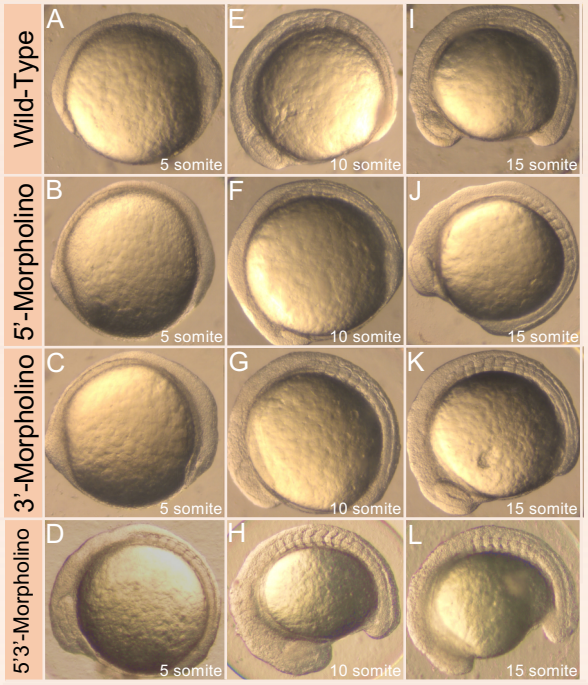

Figure 5: Embryonic development of morpholino-injected zebrafish.
A-D. 5 somite. E-H. 10 somite. I-L. 15 somite. Within 1 hour of fertilization, zebrafish embryos were injected with morpholinos targeting the 5', 3', or 5'3' ends of exon 2 of *col2a1a*. Embryonic development was imaged from 11 hpf to 24+ hpf.

Table 2: Total Phenotypes across Injections compared to WT

Phenotype	WT Controls	5' 1.5ng	5' 3.0ng	5' 4.5ng	3' 1.5ng	3' 3.0ng	3' 4.5ng	5'3' 1.5ng	5'3' 3.0ng	5'3' 4.5ng
Normal	470	70	15	26	74	10	11	68	19	27
Abnormal Tail	1	2	3	10	2	8	9	4	49	17
Edema	2	3	8	67	5	11	11	6	39	34
Abnormal Tail & Edema	1	0	35	20	1	58	99	2	52	60
Totals	474	75	61	123	82	87	130	80	159	138

Identification of CRISPR-Cas9 amenable zebrafish

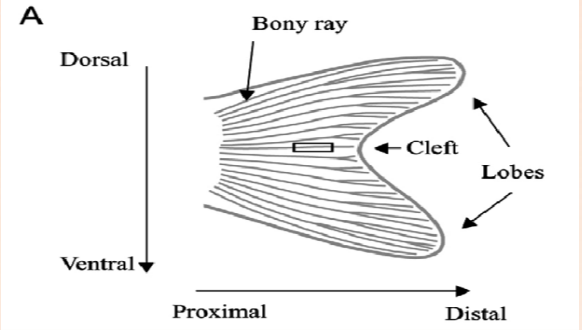

Figure 6: Diagram of Zebrafish caudal fin used during tail fin clip protocol.

Currently, our lab is using the CRISPR-Cas9 system to gene edit out the second exon from the *col2a1a gene.* Our protocol, as indicated by the figure above, begins will the clipping of the caudal fin of the zebrafish. After DNA extraction followed by PCR analysis, our group will use the Geneious computer program to assure our exon aligns with an older version of what our lab has previously sequenced.

Due to the obstacles of the COVID-19 virus, our lab group never reached our protocols to repress the exon 2 of *col2a1a.* First, our lab group could cause a nonsense or missense mutation in exon 2. Our second option is to splice out the exon entirely. Finally our group would inject the fish embryos using the reagents for our specific repression protocol to remove the target segment of DNA. The results could also provide insight on the fundamental basis of development of different health conditions such as Kniest Dysplasia, Hypochondrogenesis, and Stickler Syndrome in humans

CONCLUSIONS

1. Overexpression plasmids of full-length construct were unable to be passed on to stable transgenic zebrafish, suggesting that overexpression of full-length *col2a1a* may cause lethality.
2. Knockdown of the embryonic splice variant of *col2a1a* yielded a higher embryo mortality rate compared to the wild type embryos, suggesting that loss of this specific splice variant of *col2a1a* can interferes with organogenesis.
3. Zebrafish with reduced embryonic splice variant of *col2a1a* present with increased tissue around the tail, heart edema, and curvature of the tail.

FUTURE WORK

1. Identifying stable transgenic zebrafish with overexpressed constructs of varying lengths of *col2a1a.*
2. Determine if genes downstream of BMP signaling are disrupted when the embryonic splice variant of *col2a1a* is knocked down or overexpressed.
3. Further investigate the repression protocols of the CRISPR-Cas9 project.

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