Impaired migration of primordial germ cells and ovarian development in *adamts9* knockout zebrafish

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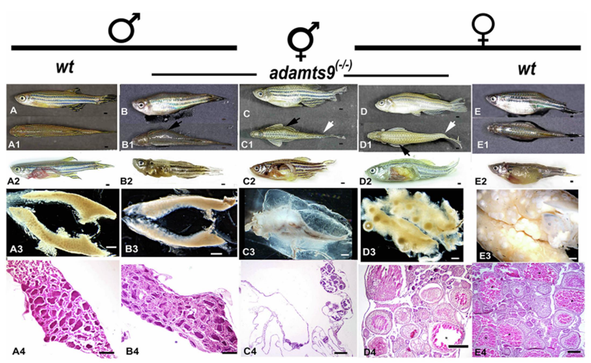
INTRODUCTION

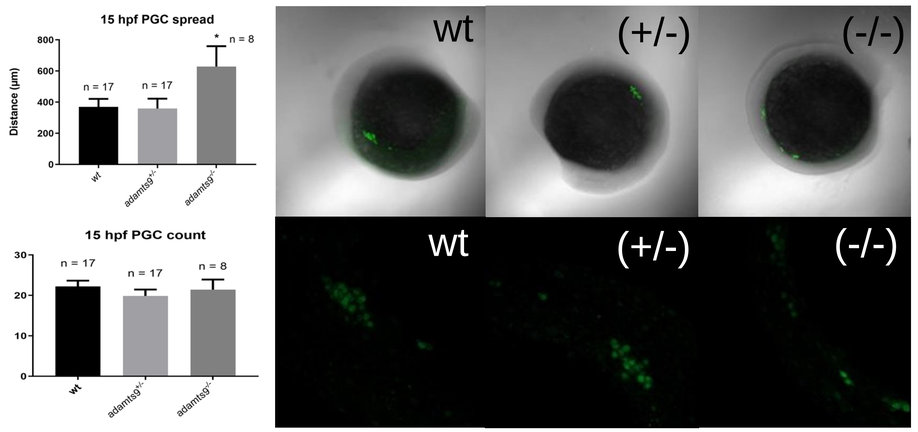
ADAMTS9 (a disintegrin and metalloproteinase with thrombospondin type-1 motif, member 9) is a highly conserved metalloproteinase that cleaves extracellular matrix substrates and signaling molecules, and may be important for organ formation and tissue remodeling. However, embryonic lethality in *adamts9* knockout mice has impeded our understanding of the roles of ADAMTS9 in vertebrates. Our lab developed the first adult, vertebrate *adamts9*$^{-/-}$ mutant model in zebrafish (*Danio rerio*), and found that *adamts9*$^{-/-}$ zebrafish have a heavily male biased sex ratio, and abnormal ovarian development (1). We hypothesize that ADAMTS9 knockout leads to mis-migration of germ cells, increase in apoptosis, and/or reduction in proliferation of primordial germ cells (PGCs), which together are the cause of biased testicular development and abnormal ovarian development. To test this hypothesis, *adamts9*$^{-/-}$ mutant zebrafish were crossed with another zebrafish line containing the transgene *vasa*::EGFP. VASA is an RNA helicase expressed exclusively in PGCs. By using the *vasa* promotor to drive GFP expression, it is possible to visualize PGCs in zebrafish embryos with a laser confocal scanning microscope. Zebrafish embryos were collected and allowed to grow before being fixed in 10% formalin for four hours at room temperature. Embryos were subsequently washed with distilled water and transferred into a PBS solution for storage at 4ºC. Fixed embryos are mounted onto slides in 1.2% low melting point agarose and then imaged by confocal microscopy.

PREVIOUS FINDINGS

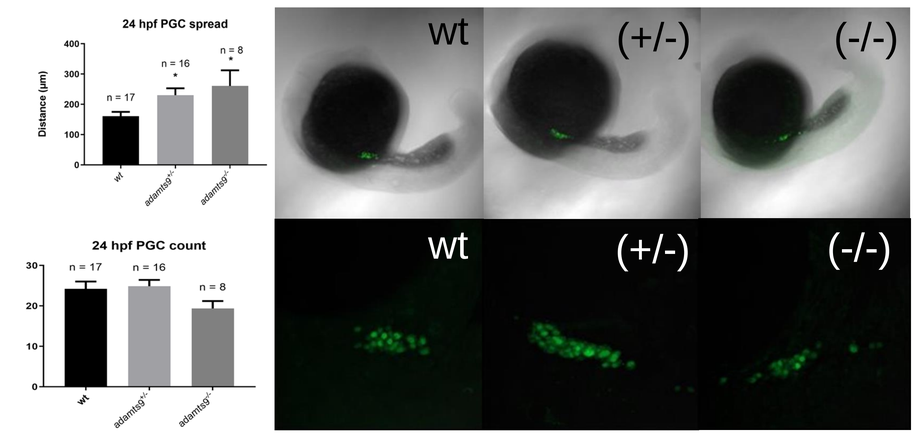


Adult *adamts9*$^{-/-}$ zebrafish have abnormal ovarian structures. Female *adamts9*$^{-/-}$ zebrafish have fewer vitellogenic oocytes, high amounts of stage I oocytes, and the ovaries were smaller overall. Interestingly, some individuals are intersex. They appear female on the outside, but contain an empty membranous organ instead of fully formed ovaries (1).

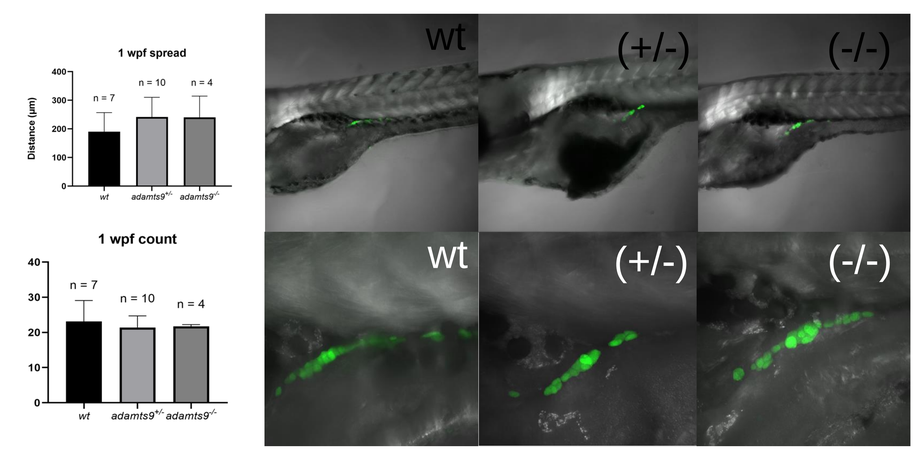
RESULTS



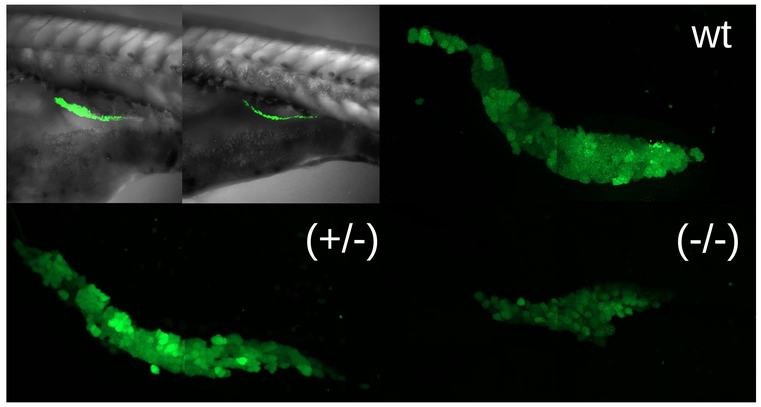
Result 1: *adamts9*$^{-/-}$ zebrafish at 15hpf show significant delay of PGC migration. No difference was detected in the amount of cells present at this time.



Result 2: *adamts9*$^{-/-}$ zebrafish at 24hpf still show delay in PGC migration. Interestingly, the *adamts9*$^{+/-}$ did show some divergence from the WT in the spread of PGCs but still was not as drastic as *adamts9*$^{-/-}$.



Result 3: *adamts9*$^{-/-}$ show relatively normal PGCs by 48hpf, and persists as normal through 1wpf. The early impairment of PGC migration disappears by 48hpf, and the PGCs are able to successfully migrate to the eventual sites of the gonad. Through the first week of development, not much further modification has happened to the larval gonad.



Result 4: Preliminary analysis of the larval gonad at 2wpf shows smaller amounts of PGCs present. The relative position of the PGCs and developing gonad in 2wpf larva is shown in the top left. In normal gonadal development, significant proliferation occurs between 1wpf and 2wpf. The adamts9$^{-/-}$ zebrafish appear to have lower amounts of PGCs.

DISCUSSION

Mutant zebrafish embryos at 15 hours post fertilization (hpf) and 24 hpf showed significant delay in PGC migration. However, by 48 hpf the mutant phenotype had recovered to resemble wildtype embryos and PGC levels remained about the same as wildtype embryos for the first week of development. While the PGC migration was shown to be impaired, the critical time period when ovarian development was impaired is still unknown.

Preliminary analysis suggests that 2 wpf larval gonad in *adamts9*$^{-/-}$ fish have reduced proliferation of PGCs. Future work will examine 4 wpf larva, as well as 45 dpf and 90 dpf.

REFERENCES

(1) *Carter, N.J., Roach, Z.A., Byrnes, M.M., and Zhu, Y. (2019). Adamts9 is necessary for ovarian development in zebrafish. General and Comparative Endocrinology. 277, 130-140. DOI: 10.1016/j.ygcen.2019.04.003*

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