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Abstract:
Somatosensory neurons enable appropriate responses to environmental stimuli, thereby allowing us avoid pain and danger [1]. Different populations of these neurons respond to various types of sensory stimuli such as touch, temperature, chemical, and pain [2]. Somatosensory neurons grow by branching through the skin, and their goal is to maximize coverage [3]. Neuronal migration and axonogenesis, which are vital to this proper neuronal development require the activity of many cellular components – including cytoplasmic microtubules [7]. Therefore, proteins in charge of cytoskeletal regulation are thought to help control and guide neuron shape [4,5].

Many genes are involved in the regulation of neuronal structures and these genes have the potential to mediate interactions between somatosensory neurons and the environment. One such gene family is the tubulin polymerization promoting proteins (tppps) which contains tppp, tppp2, and tppp3 (figure 1). Tppp proteins function in the polymerization of the microtubule tubulin in vitro [6]. Axonogenesis, the extension of a single immature process and its subsequent differentiation into an axon, is controlled by the polarization of microtubules [7]. Neuron polarization and neurite outgrowth are also tightly controlled by microtubule stabilization and acetylation [13], and altered microtubule stability underlies the dynamic nature of growth and retraction processes in neurons [7]. The extensive rearrangement of cell shape that occurs during this migration is mediated by changes in the cytoskeleton [8]. Previous research has shown that microtubule stabilization is sufficient to induce axon formation and that moderate microtubule destabilization selectively reduces the formation of minor neurites [12]. Therefore, tppp2 may have an essential role in regulating the shape of somatosensory neurons via tubulin stabilization.

While the biochemical role of tppp is fairly well characterized [6], the role of its paralog tppp2 is relatively unknown, though, tppp2 has been shown to be expressed in sensory neurons [9]. Injection of antisense morpholinos targeting zebrafish tppp2 reduced axon extension in Rohon Beard neurons (RB) and motorneurons, suggesting that tubulin polymerization may be crucial for proper axon growth [9]. **Since tppp2 functions in neuronal stabilization, I hypothesize that a knockout of the tppp2 would lead to decreased stabilization of tubulin and thus decrease the number of TGG projections formed**.

My research goal has been to understand the role of tppp2 in zebrafish somatosensory system development and somatosensory reception. Previously I have worked to identify founders for CRISPR/Cas9-induced mutations of tppp2, performed the husbandry to cross these mutations to homozygosity. I then have prepared assays to evaluate and analyze loss -of function mutations focusing on development of neuronal shape, and function of these neurons in detecting sensory stimuli.

Via confocal imaging of acetylated tubulin antibody tagged neurons, I have investigated developmental changes to trigeminal ganglion peripheral projections, a population of

sensory neurons in the head, along with RB neurons in the tail (figure 2). I hypothesize that there will be a decreased number of peripheral projections in my KOs. In contrast to Aoki, I have found no apparently difference between KOs and their wild-type counterparts. I also aim to elucidate the specific function of these projections through developmental and behavioral assays of larval zebrafish. Preliminary data has shown that these tppp2 KO's may respond differently to chemical stimuli than their wild-type or heterozygous counterparts, but respond normally to thermal stimuli (figure 3).

As described above, we have been unable to replicate the findings of the tppp2 MO paper in our knockouts (KOs) [9], one hypothesis for why this is occurring is functional compensation due to gene duplication. This would mean the duplicated versions of tppp2, are able to rescue the KO [14]. Due to the potential functional compensation of our tppp2 knockouts (KOs), I will perform experiments to determine if tppp2 overexpression plays a role in the dysregulation of peripheral projection development.

These studies will provide the groundwork for determining the specific mechanisms underlying the role of microtubules in neuronal development, as well as enhance our understanding of somatosensation. Dysfunction of somatosensory neurons can cause chronic pain [11]; accordingly, improved understanding of sensory neuron development and function could enable the generation of treatments for chronic pain conditions. Furthermore, we could use this knowledge to enhance our understanding of diseases causing sensory deficits in humans.
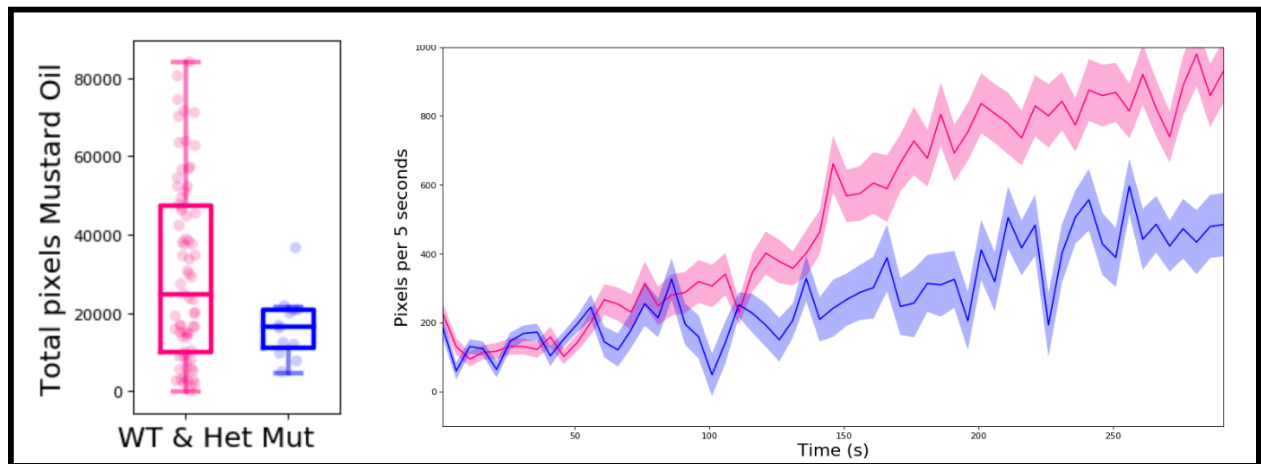
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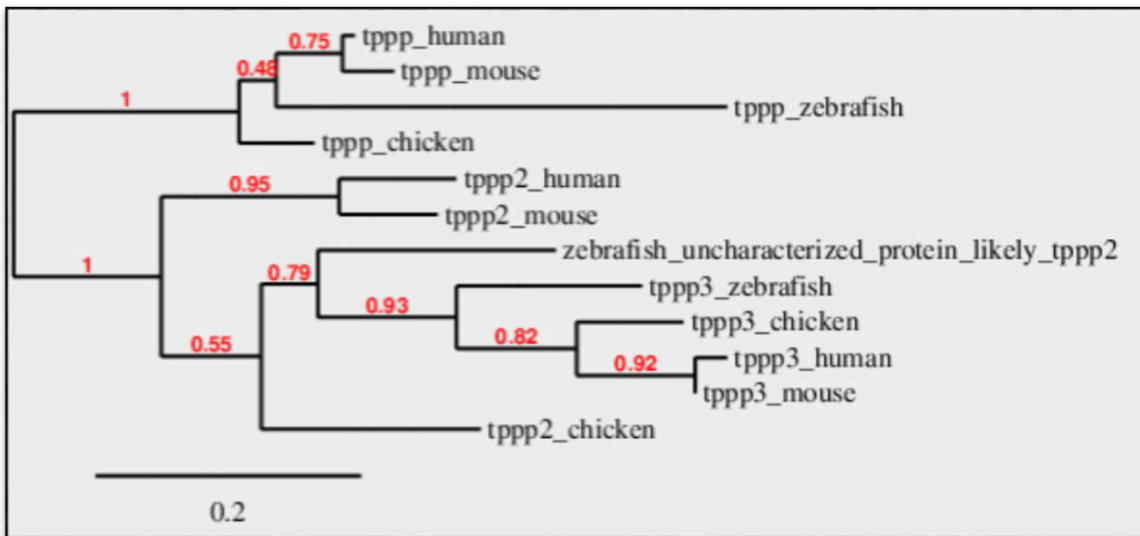
Figure 1: Phylogeny of tppp protein family in vertebrates. Tppp proteins are more related between paralogs than within species.
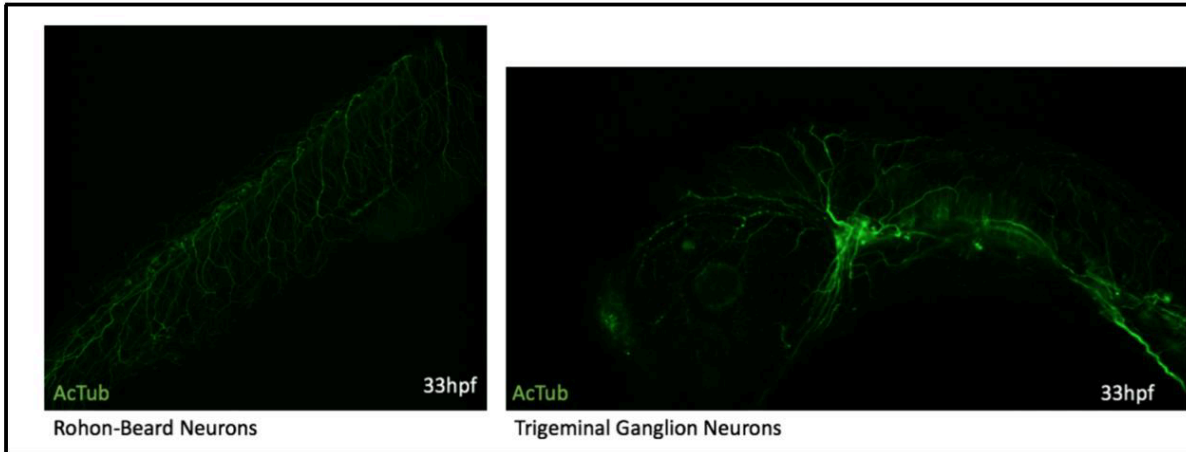
Figure 2: Acetylated Tubulin antibody staining in populations of developing sensory neurons present in larval zebrafish. Rohon-Beard Neurons are present in the trunk of larval zebrafish and Trigeminal Ganglion neurons are present in the head.

Figure 3: tppp2 Mutant larval zebrafish show decreased response to chemical (mustard oil) stimuli compared to wildtype and heterozygous fish. Pixels represent difference in motion over time analyzed, total pixels represent total differences in movement.

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