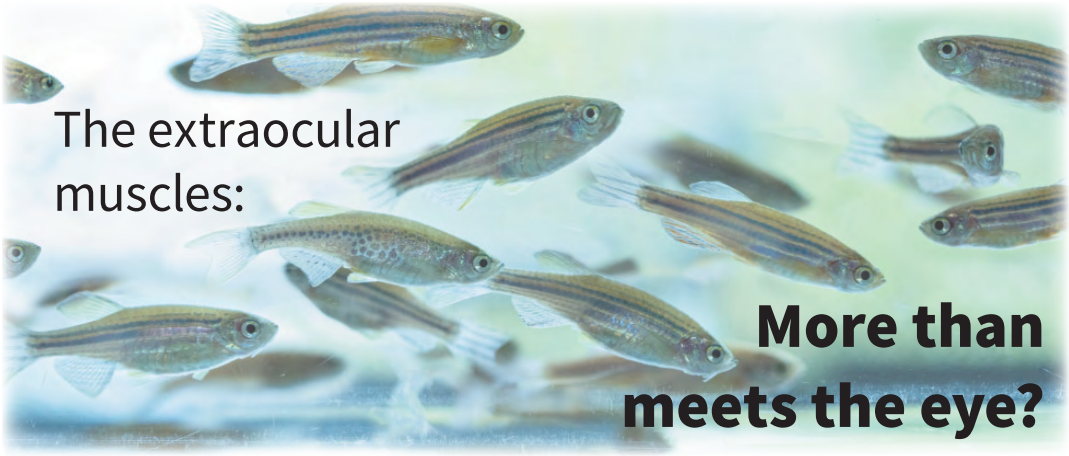

The extraocular muscles: More than meets the eye?

Nils Dennhag
Dept. of Medical and Translational Biology, Dept. of Clinical Sciences, Ophthalmology, Umeå University, Sweden

On January 26, 2024, Nils Dennhag defended his thesis "Genetic studies of zebrafish muscles – clues to protection in muscle disease" at the Dept. of Medical and Translational Biology, Umeå University, Sweden. The Ph.D. project was conducted at the Dept. of Clinical Sciences, Ophthalmology, Umeå University, Sweden. The main supervisor was Prof. Fatima Pedrosa Domellöf, Dept. of Medical and Translational Biology, Dept. of Clinical Sciences, Ophthalmology, Umeå University, and the co-supervisor was Dr. Jonas von Hofsten, Dept. of Medical and Translational Biology, Umeå University.

Key points:

- The extraocular muscles (EOMs) differ from trunk muscles in healthy and diseased states.
- The EOM transcriptome offers unique insights through its protective properties in disease.
- The EOMs' innate protective properties can be applied to ameliorate muscular dystrophies.

Muscular dystrophies are a group of diseases in which different proteins in the muscles malfunction because of either hereditary or spontaneous mutations. As a result, the myofibers (muscle fibers) gradually degenerate, leading to muscle weakness, loss of ambulance, and premature death, most often due to cardiac or respiratory failure. Strikingly, the extraocular muscles (EOMs) are not affected by these diseases and function well throughout the disease progression. This trait engenders several interesting research areas, including the differences between EOMs and other muscles of the body as well as how the gene regulation of EOMs in disease evades functional decline.

Our research explored EOMs in the zebrafish model utilizing CRISPR/Cas9 to induce genome-wide mutations in muscle-related genes, such as *obscurin*, *desmin*, and *dystrophin*. Special attention was placed on studying the protein desmin and zebrafish *desmin* mutants, as this model offers the opportunity for prolonged longitudinal studies throughout disease progression. By sequencing the transcriptomes of EOMs and trunk muscles in *desmin* mutants and controls, we were able to partially decipher the cause of EOMs' innate resistance in muscle disease—the upregulation of the gene *fhl2*. We determined that *fhl2* protects EOM myofibers by preventing their degeneration and hypertrophy (pathological enlargement).

The *fhl2* gene is not expressed in most body muscles in zebrafish or humans; however, through the ectopic expression of *fhl2* specifically in muscle tissue, we observe substantial improvements in survival in the most severe muscle dystrophy model of Duchenne muscular dystrophy (DMD). In addition to better survival, zebrafish expressing *fhl2* in their body muscles had improved muscle structure, increased muscle innervation and regeneration, and better swimming capabilities compared with sibling controls (**Figure 1**). In summary, our research showed that EOMs' innate resistance can be utilized as a road map to discovering strategies to ameliorate muscular dystrophies and allow patients to survive them. Furthermore, we believe that our research has the potential for human applications, as human EOMs also express *fhl2*. We believe that we are just scratching the surface of what is possible with our newly gained knowledge regarding EOMs, and we intend to continue to build on this understanding.

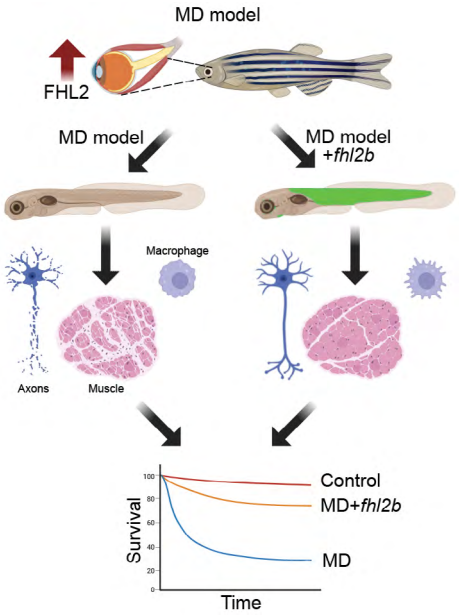

Figure 1. Summary of the results of the thesis. The fhl2 *gene is upregulated in the EOMs of* desmin-, plectin-, *and* obscurin-*knockout zebrafish. Muscle-specific ectopic overexpression of* fhl2 *rescues the integrity of myofibers, axons, and neuromuscular junctions and quickens the pace of muscle regeneration in zebrafish lacking dystrophin. This leads to increased swimming capabilities and extended survival in zebrafish lacking dystrophin. From Dennhag N. Genetic studies of zebrafish muscles: clues to protection in muscle disease [Internet] [PhD dissertation]. [Umeå]: Umeå University; 2023. (Umeå University medical dissertations).*

Future Directions:

- Our data indicate that adaptations to muscle weakness and changes are fine-tuned in EOMs, but the precise mechanisms of this fine-tuning remain to be studied.
- Small molecule studies will determine whether *fhl2* levels can be manipulated by existing drugs to treat muscular dystrophies.

References

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