Generating Zebrafish model for studying the mechanism underlying the rare genetic disease: N-glycanase deficiency

Mesika, B.Sc[1,2], Golan Nadav, PhD[1,2], Chen Shochat, PhD[2], Limor Kalfon, PhD[1], David Karasik, PhD[2], Tzipora C. Falik-Zaccai, MD[1,2]

[1]Institute of Human Genetics, Galilee Medical Center, Nahariya. [2] Azrieli Faculty of Medicine, Bar Ilan University, Safed.

Background

NGLY1 is an enigmatic enzyme with multiple functions across a wide range of species. It catalyzes the de-glycosylation of misfolded glycoproteins, mediates signaling pathways and is involved in regulation of mitochondrial physiology. In humans, pathogenic genetic variants in *NGLY1* are linked to a variable phenotype of neurological dysfunction, abnormal tear production, and liver disease as part of the rare autosomal recessive disorder- N-glycanase deficiency with less than 100 patients reported worldwide.

Aim

We aim to generate a novel zebrafish model, for *NGLY1* deficiency, to characterize phenotypes and study the pathophysiologic mechanisms involved in this rare disorder.

Methods and Results

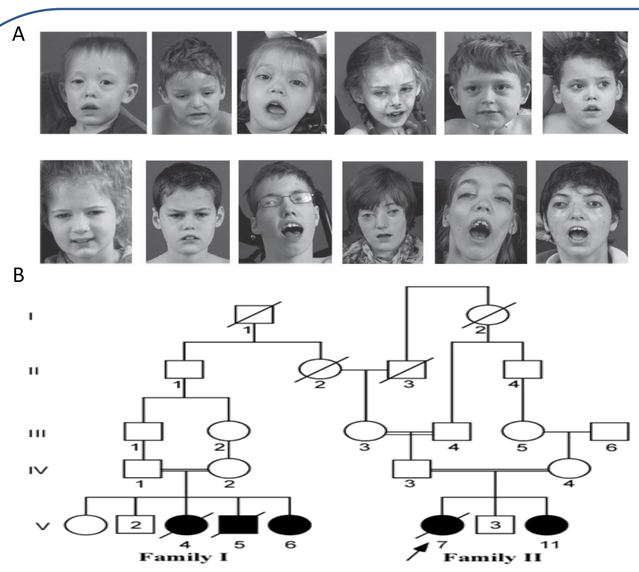

Fig.1: Pedigree and clinical features. (A) reported patients with NGLY1 deficiency. (B) Pedigree of the extended Druze family presenting a pathogenic genetic variant in *NGLY1.*

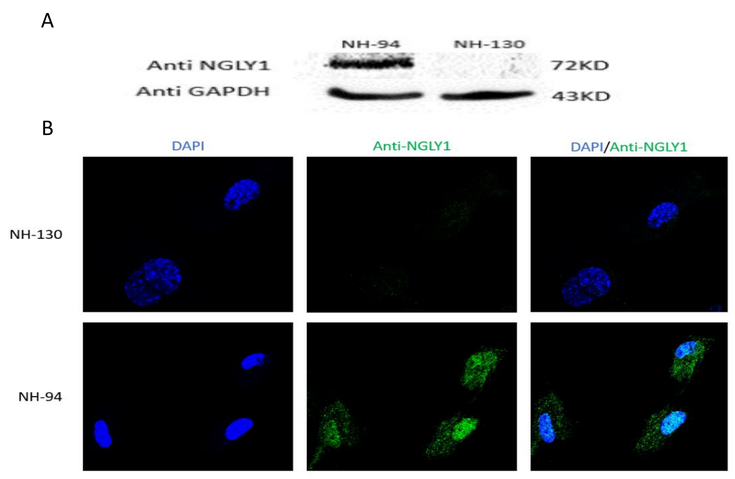

Fig.2: NGLY1 protein levels are reduced in patients. (A) WB analysis of fibroblasts from a patient (NH130) and healthy control (NH94). (B) Immunofluorescence staining. Both methods show reduced NGLY1 protein in fibroblasts from NH130.

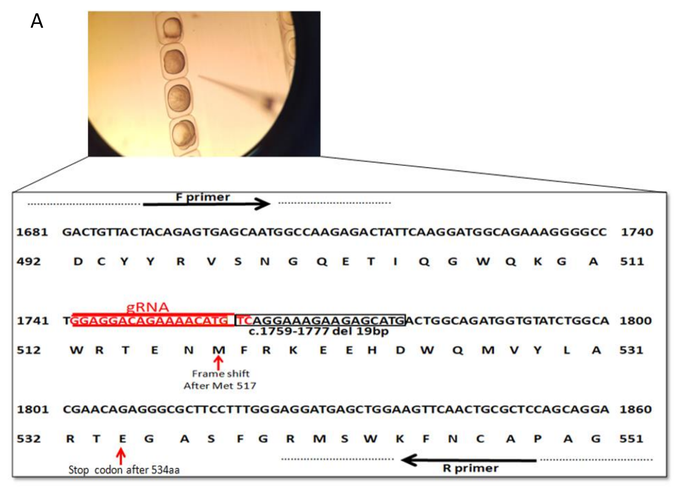

Fig.3: Schematic representation of the *ngly1* gene targeting in zebrafish.

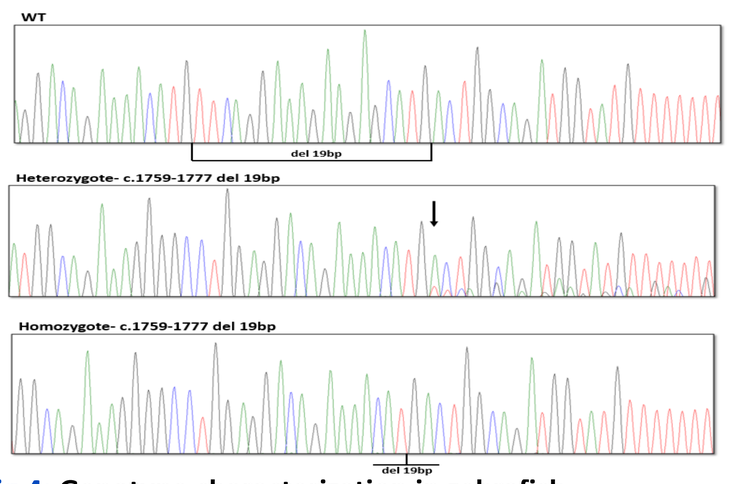

Fig.4: Genotype characterization in zebrafish. Representative Electrophrograms indicating the sequences of WT, heterozygote and homozygote.

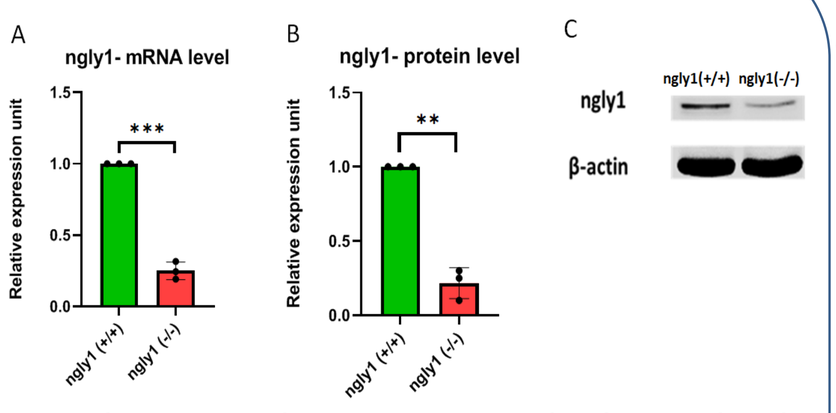

Fig.5: *ngly1* mRNA and protein expression levels are reduced in *ngly1* KO zebrafish. (A) qPCR analysis quantification of ngly1 mRNA levels. (B) WB analysis quantification of ngly1 protein levels. (C) WB analysis with an antibody against ZF ngly1.

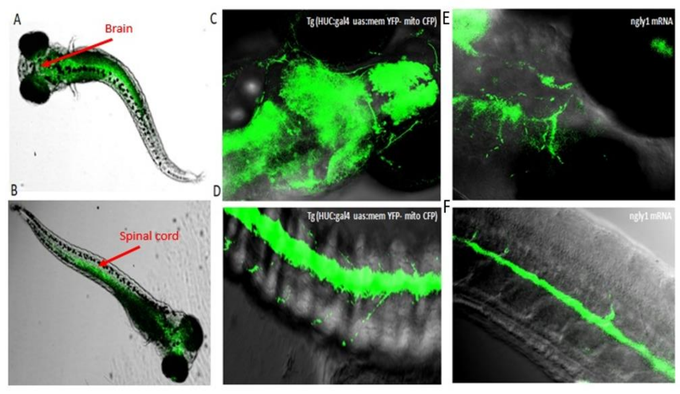

Fig.6: *ngly1* expression in the nervous system (green=nervous) of WT zebrafish . (A) Lateral view and (B) ventral view. (C) Brain (D) Spinal cord of Tg(HUC:gal4 uas:mem YFP-mito CFP). (E) Brain (F) Spinal cord of WT larvae.

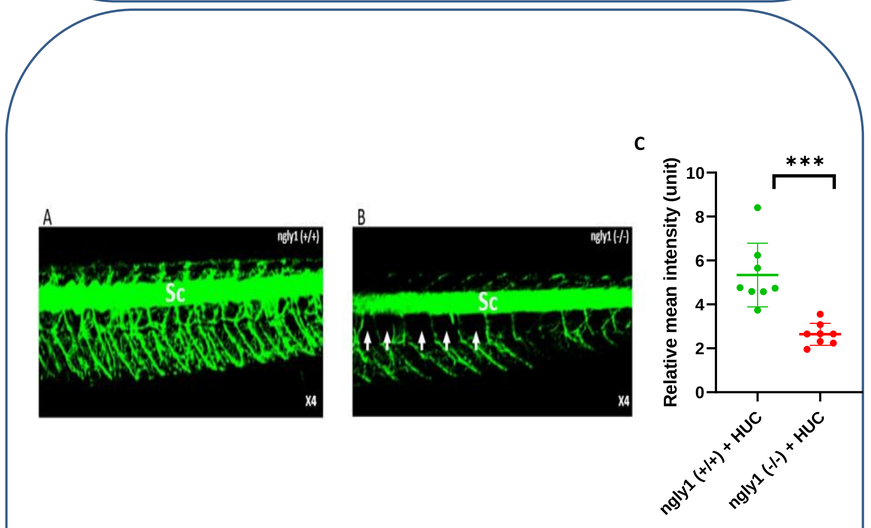

Fig.7: Confocal imaging of spinal cord and peripheral axons. (A*) ngly1(+/+)* ZF control and (B) *ngly1(-/-)* ZF knockout. Sc=spinal cord, white arrows represent the loss of axon fibers. (C) Quantification analysis of the peripheral nervous system.

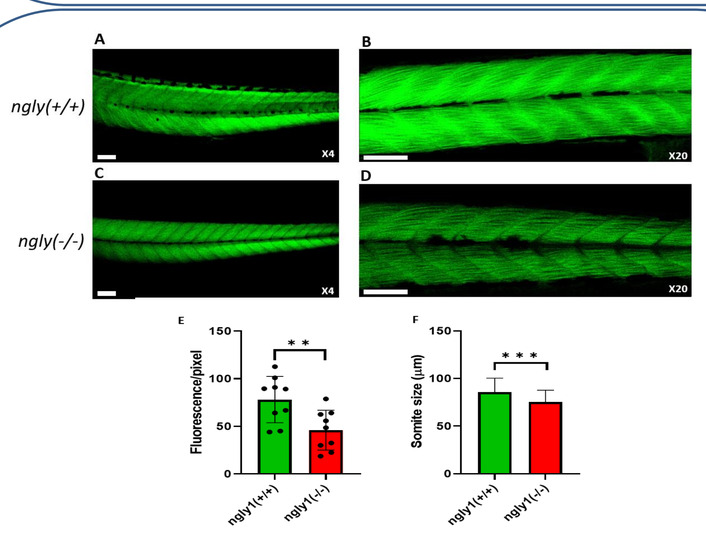

Fig.8: Muscle structure morphology. (A-B) Representation of *ngly1 (+/+)* and *ngly1 (-/-)* (C-D) Phalloidin staining for actin(A-X4 and B-X20). (E) Quantification analysis of the Phalloidin fluorescence. (F) Mean size of somite (µm). Scale bars - 50µm.

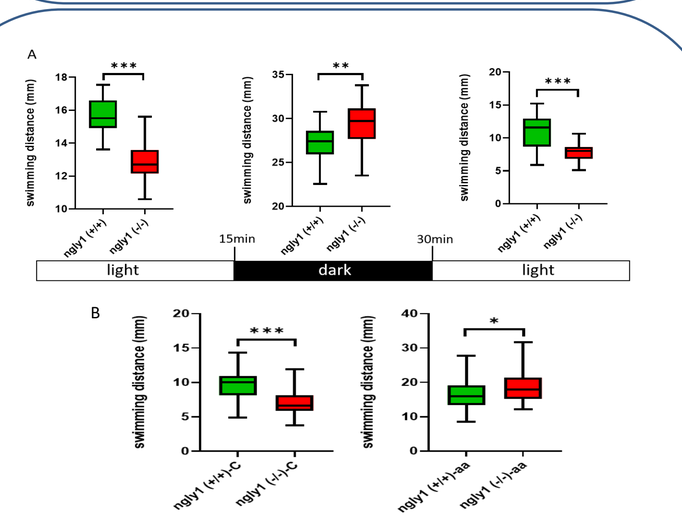

Fig.9: Behavioral phenotypes. (A) locomotion behavioral analysis in light and dark environment. (B) Sensitized acid aversion assay: quantification analysis of swimming distance after acetic acid treatment. aa: acetic acid.

Conclusions

- **We generated a viable zebrafish model for NGLY1 deficiency.**
- **NGLY1 is expressed in the nervous system of the WT zebrafish.**
- **Nervous system morphology analysis has shown significant loss of axon fibers in the mutant fish compared to the WT controls.**
- **Significant difference was observed in muscle morphology between the two genotypes - *ngly1(-/-)* and *ngly1(+/+).***
- **Locomotion behavior analysis has shown hypersensitivity of the larval *ngly1(−/−)* fish during stress conditions compared to their WT siblings.**