Common mechanisms between SCA36 and C9ORF72 ALS/FTD: Insights from a novel zebrafish model

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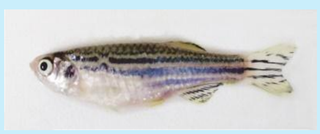
Introduction

SCA36 is a dominant inherited neurodegenerative disorder characterized by:
- Gait imbalance.
- Hearing loss.
- Motoneuron involment.

Fig.1: Distribution of SCA36 in Spain and Japan.

Expansion >30 repeats GGCCTG in *NOP56* gene



Zebrafish models:
- Widely used.
- Easy to assess CNS defects.
- Easy to perform behavioral studies.

• Loss of function mechanisms

nop56 KO zebrafish model (Quelle-Regaldie *et al.*, 2022)

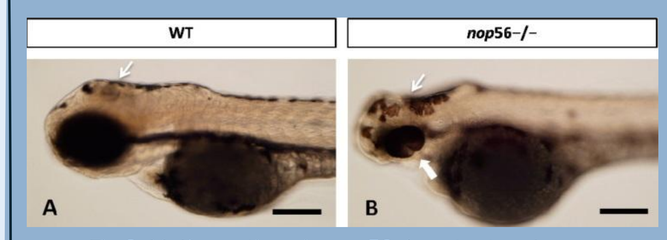


Fig.2: Malformations in *nop56-/-* mutant embryos vs WT at 3.5dpf.

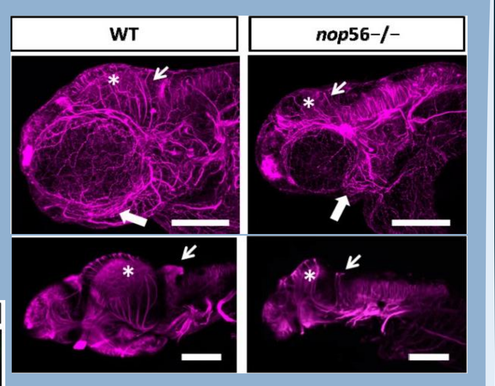


Fig.3: anti-tubulin revealed neuronal malformations in 3.5dpf *nop56-/-* embryos.

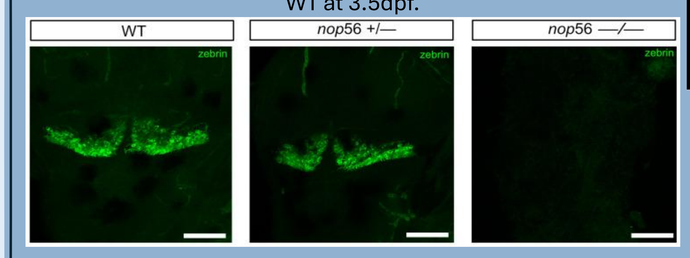


Fig.4: anti-zebrin revealed lack of Purkinje neurons in *nop56-/-* larvae at 4dpf.

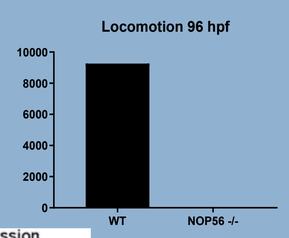


Fig.5: *nop56-/-* embryos are unable to swim at 96hpf.

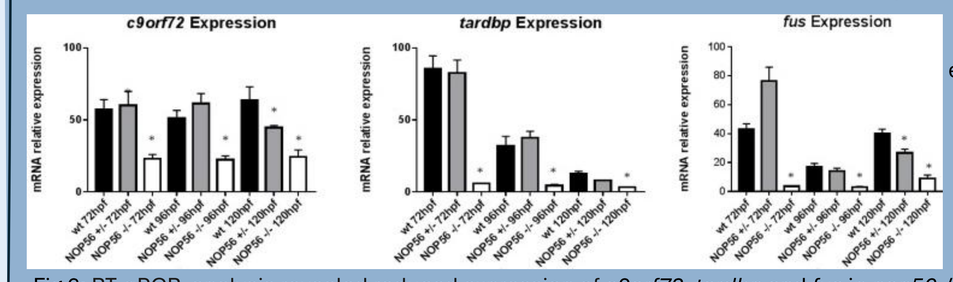


Fig 6: RT-qPCR analysis revealed reduced expression of *c9orf72, tardbp* and *fus* in *nop56-/-* mutants.

• Gain of function mechanisms

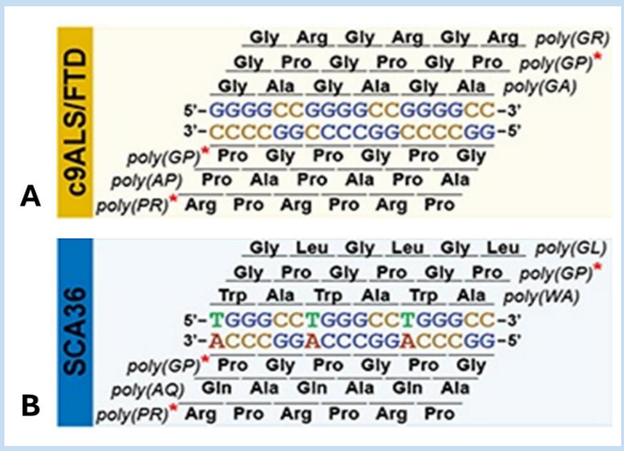
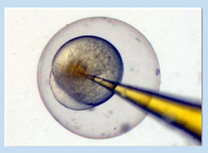


Fig.7: Dipeptides formed by RAN translation in A. Amyotrophic Lateral Sclerosis caused by mutations in *C9ORF72* (C9/ALS) and B. SCA36. Adapted from McEachin *et al.*, 2020.

ALS and SCA36 shared poli-GP DPR

SCA36 poly-GP GFP and C9/ALS poly-GP GFP injected in one-cell zebrafish embryos



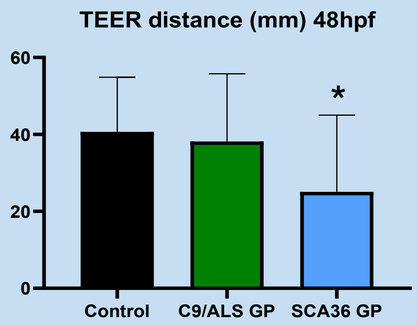
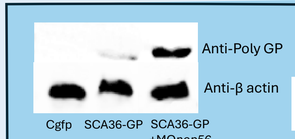


Fig.8: Touch-evoked escape (TEER) activity is reduced in embryos injected with SCA36 poly-GP at 48hpf but not in embryos injected with C9/ALS poly-GP meaning that only SCA36 poly-GP is toxic.



• Combination of mechanisms

Injection of nop56 MO (subphenotypical dose) + SCA36 poli-GP

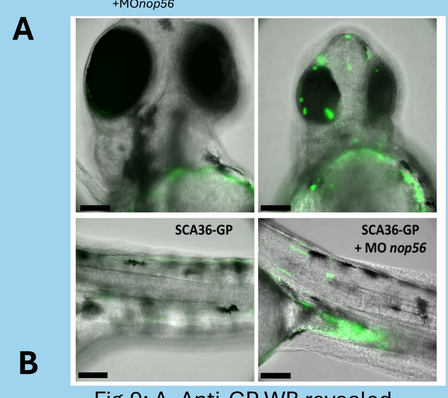


Fig.9: A. Anti-GP WB revealed accumulation of poly-GP under *nop56* knockdown at 48hpf. B. GFP fluorescence imaging revealing poly-GP aggregates in SCA36-GP+MO*nop56* embryos but not in SCA36-GP alone.

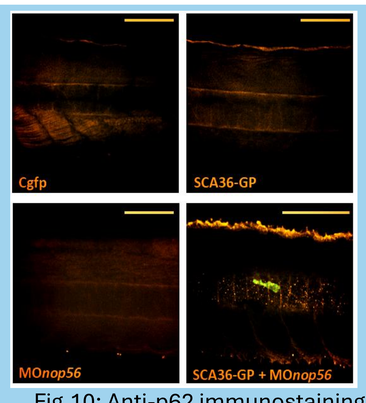


Fig.10: Anti-p62 immunostaining revealed p62 aggregation in musculature and notochord of SCA36-GP+ MO*nop56* embryos at 48hpf.

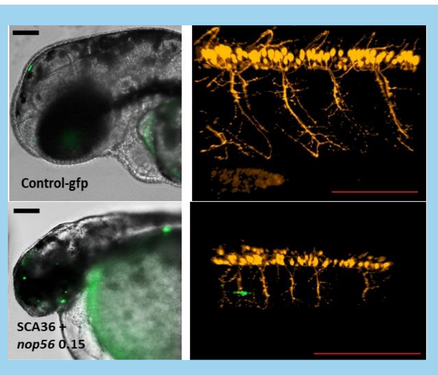


Fig.11: Aggregation of poly-GP inclusions in brain of 48hpf SCA36-GP+ MO*nop56* embryos and motoneuron malformations.

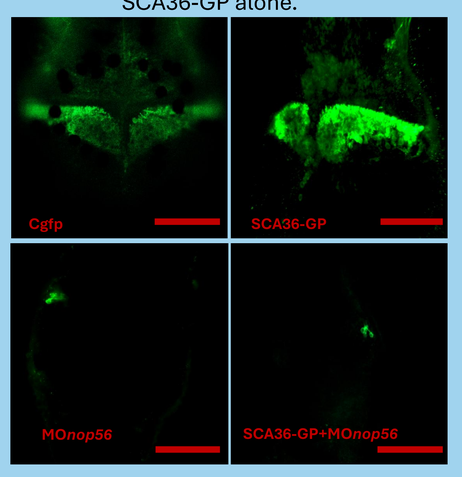


Fig.12: MO*nop56* and SCA36-GP+MO*nop56* groups show incomplete cerebellum with the presence of only a few Purkinje cells at 4dpf.

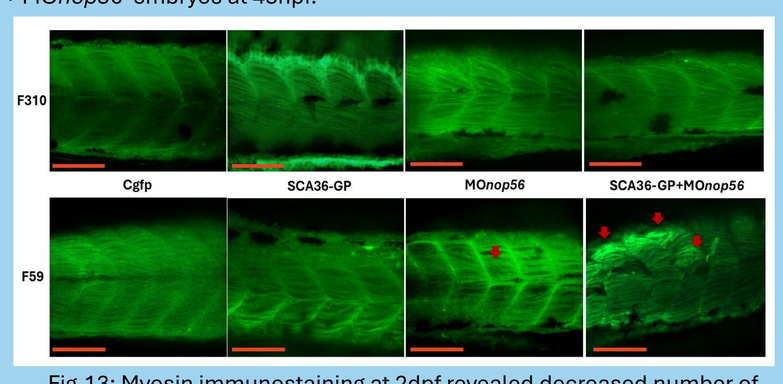


Fig.13: Myosin immunostaining at 2dpf revealed decreased number of myosin light chain (F310) and myosin heavy chain showed disorganized fibers (F59) in MO*nop56* and SCA36-GP+MO*nop56* embryos.

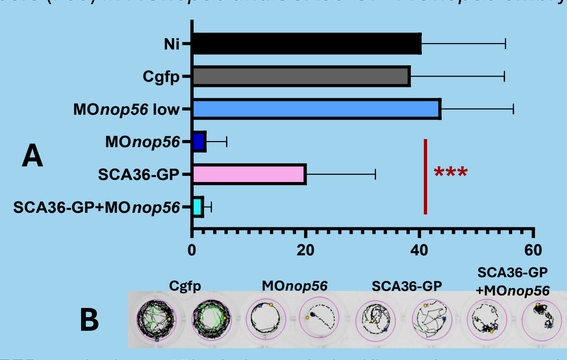


Fig.14: A. TEER analysis at 48hpf showed significant locomotor dysfunction in SCA36-GP, MO*nop56* and SCA36-GP+MO*nop56* groups. B. Representative wells from total locomotion at 4dpf in the 3 affected groups.

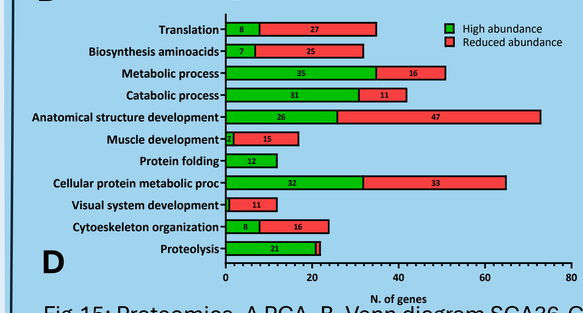


Fig.15: Proteomics. A PCA. B. Venn diagram SCA36-GP vs SCA36-GP+ MO*nop56*. C. Shared most affected KEGG pathways. D Pathways affected in SCA36-GP+ MO*nop56*.

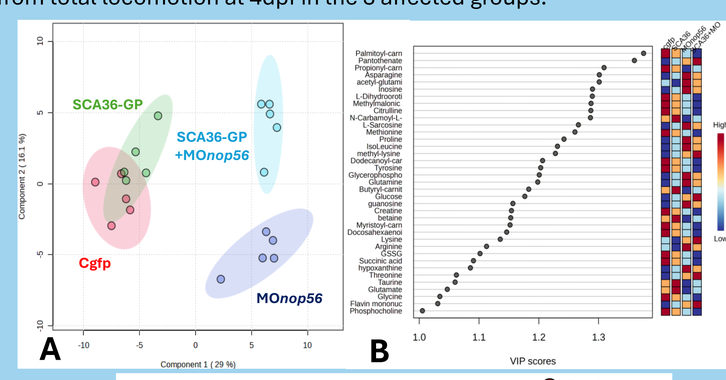


Fig.16: Metabolomics. A PCA. B. VIP score >1 highligh the most relevant metabolites. C. Integrated analysis of the proteomic and metabolomic pathways more altered in combined model.

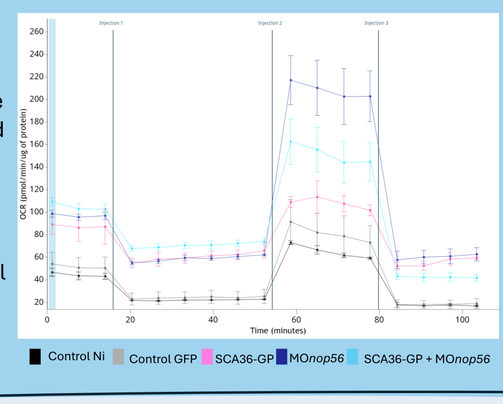


Fig.17: Seahorse análisis revealed increased mitocondrial respiration in MO*nop56* and combined model at 2dpf.

Conclusions:

- SCA36 could be caused by combination of gain and loss of function mechanisms.
- Poly-GP aggregates only under haploinsufficiency and caused neurodegeneration and muscular defects.
- Combined model recapitulate key features of the human disease
- SCA36 and ALS seem to share pathways including ribosomal homeostasis, proteolysis and mitochondrial dysfunction.

References

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