Establishing a Zebrafish model for Osteoporosis

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Introduction

- The low-density lipoprotein receptor-related protein 5 (***lrp5***) gene is known for its involvement in bone metabolism in humans. It is unclear whether it is involved in bone function starting early in life.
- The **purpose of our study** was to investigate whether knocking out *lrp5* gene will affect early bone development and create osteopenia.
- We created a CRISPR *lrp5* knockout zebrafish and phenotyped its skeleton at larval stages.

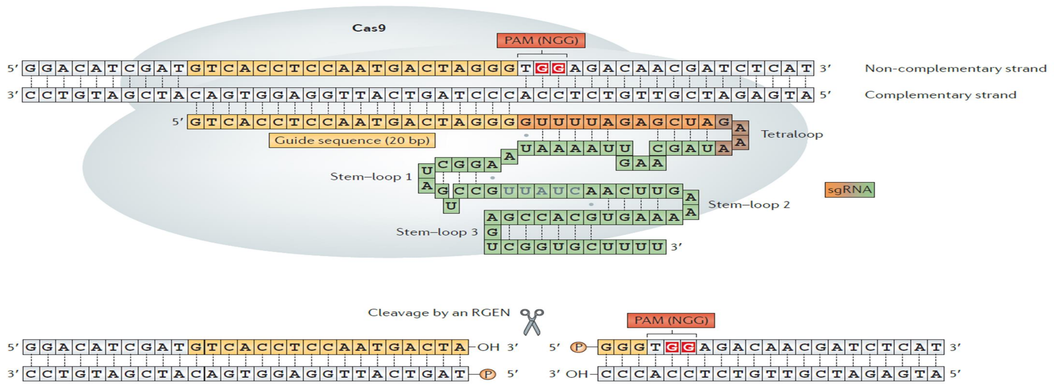

Figure 1. Scheme of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) system for genetic engineering (Kim and Kim 2014 *Nature Reviews*).

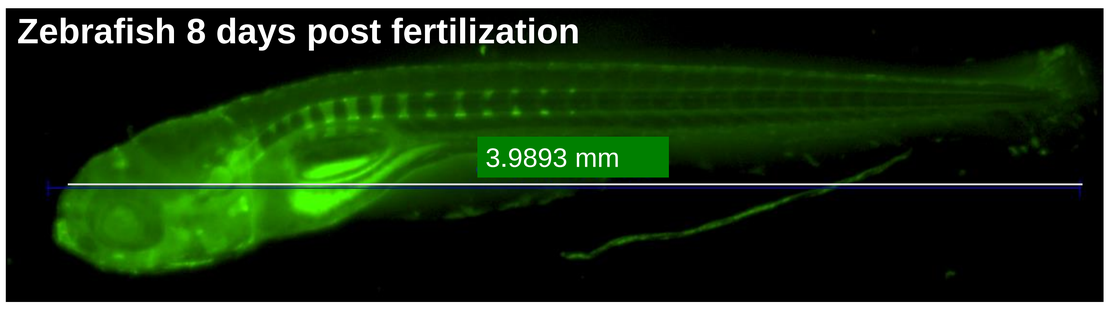

Figure 2. Fluorescent calcein staining of 8 dpf fish shows mineralized vertebrae. Length indicated in mm.

Materials and methods

- CRISPR-Cas9 technology was used to create guide RNA for *lrp5* gene and Cas9 mRNA (***fig. 1***).
- Zebrafish embryos (F0) at 1-cell stage were injected with CRISPR, grown to adulthood (3 months) and mated to obtain progeny (F1).
- At 8, 10 and 13 days post fertilization (dpf) F1 zebrafish were stained with calcein and visualized under fluorescent microscope (***fig. 2***).
- Number of mineralized vertebrae was compared between wild type and *lrp5* mutated zebrafish, adjusted for length.

Results

- No polymorphism was found in lrp5 guide RNA target site (***fig. 3***)
- F1 progeny of *lrp5* CRISPRed zebrafish had knockout mutations caused by frameshifts (***fig. 4***).
- Significant difference in the number of mineralized vertebrae between WT and *lrp5* mutated ZF was found only at 8 dpf (***fig. 5,6***).

Number of mineralized vertebrae in wild type and *lrp5* mutated zebrafish

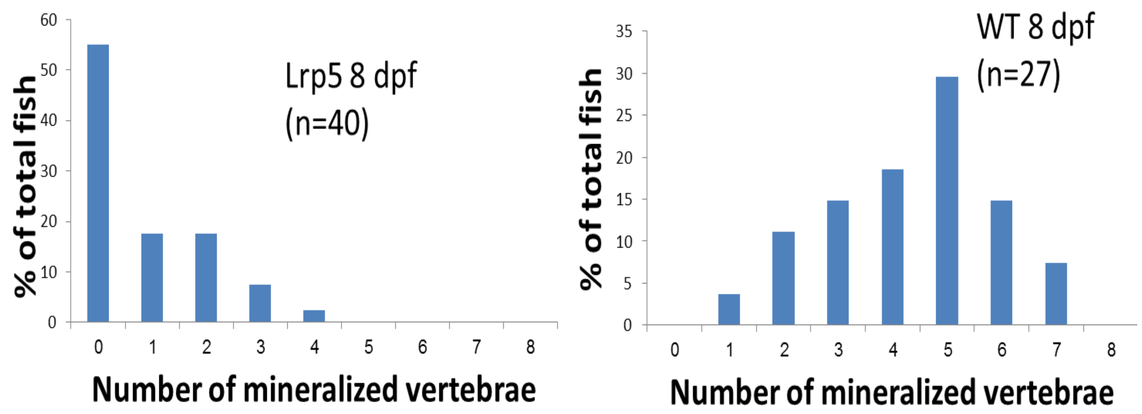

Figure 5. Number of mineralized vertebrae in *lrp5* mutated (left) and wild-type (WT, right) zebrafish, age 8 dpf.

lrp5 CRISPR-Cas9 target site

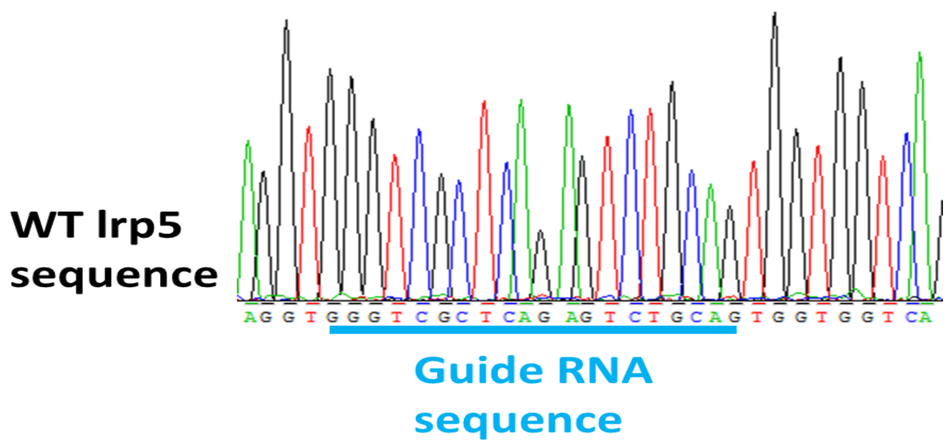

Figure 3. No polymorphism found in zebrafish lrp5 guide RNA target site for CRISPR-Cas9, allowing hybridization of the guide RNA to its target.

Zebrafish *lrp5* knockout mutation – Val53fs

WT	GAG	TCT	GCA	GTG	GTG	GTC	AGT	GAT
	Glu	Ser	Ala	Val	Val	Val	Ser	Asp
Mut	GAG	TCT	GTG	GTC	AGT	GGT	GGT	CAG	TGA	ins 4bp
	Glu	Ser	val	Val	Ser	Gly	Gly	Gln	stop	

Figure 4. Sequencing of DNA from lrp5 CRISPR-Cas9 injected zebrafish shows a knockout mutation in *lrp5* gene in F1 progeny (WT-wild type, Mut- mutated).

Number of mineralized vertebrae at 8, 10, 13 dpf in mutants and WT

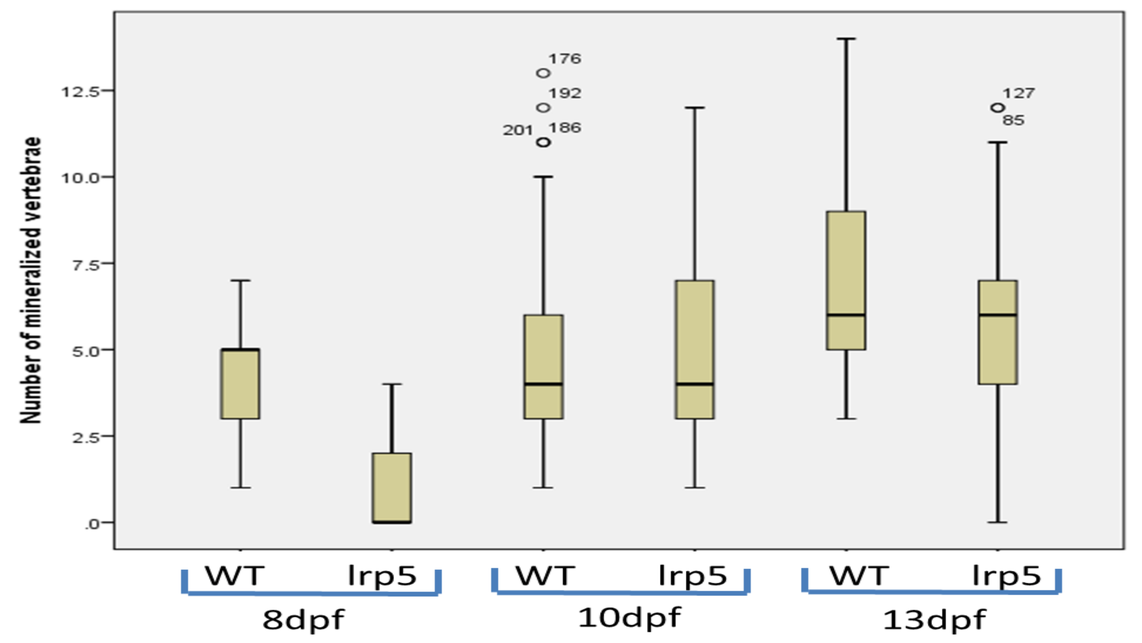

Figure 6. Significant difference was found in the number of mineralized vertebrae (but not length) between WT and *lrp5* knockout ZF only at 8 dpf.

Conclusions

- Zebrafish is highly amenable to CRISPR-Cas9 mutagenesis.
- We established *lrp5* knockout in zebrafish
- We applied a calcein staining assay to screen *lrp5* zebrafish mutants for bone development (ossification).
- Our results indicate that *lrp5* knockout influences very early stages of bone development (mineralization).