Background/Introduction: In humans, loss-of-function mutations in Low-density Lipoprotein Receptor-related Protein 5 (*LRP5*) cause Osteoporosis-Pseudoglioma syndrome, a low bone mass disorder, while missense mutations have been observed in individuals with high bone mass. LRP5 is a co-receptor of Wnt-signalling pathway, which controls expression of genes involved in osteogenesis. Like in humans, the zebrafish (*Danio rerio*) skeleton forms either by using cartilage scaffold as a template, or directly (without cartilage scaffold). Genetic determinants that control bone formation are highly conserved between zebrafish and mammals, which was supported by the finding that *lrp5* is required for neural crest cells migration and cranial skeleton morphogenesis of *lrp5* crispants (Willems et al., 2015). However, the systemic effect of lrp5 deficiency and shared functional roles within vertebrates remain unknown.

Purpose: We therefore generated *lrp5* knock-out zebrafish which allowed us to follow skeletogenesis from larval to adult stages in the zebrafish and directly compare its role in regulating skeletal differentiation and development.

Methods: *lrp5* stable knock-out was generated by CRISPR-Cas9 genomic editing. Incrossed carrier progeny was analyzed for mineralized bone matrix and cartilage by Alizarin Red and alcian blue staining across developmental stages. Adult (6 mo) progeny was also scanned by micro-CT Bruker SkyScan 1172 for skeleton phenotype.

Results: Alizarin red staining revealed that notochord mineralization at 7d and 13d of development is delayed and the total mineralization level of 27d and adult's skeleton is lower in *lrp5* knock-out fish. Further, micro-CT scanning of adults demonstrated malformations in the cranial skeleton of *lrp5* mutants, with accumulating fractures observed in parasphenoid and mandible bones. Additionally, we observed decreased whole body bone mineral density in adult mutants.

Conclusion(s): In summary, our mutant analysis demonstrates that lrp5 is important for skeletal differentiation, as its absence results in delayed mineralization throughout the zebrafish axial skeleton at early stages, consequently resulting in deformation of neuro- and viscerocranium.

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P221 (ND)

Variation of shape, bone structure and mineralisation of vertebral centra in young adult chihuahua, a zebrafish model for human classical osteogenesis imperfecta

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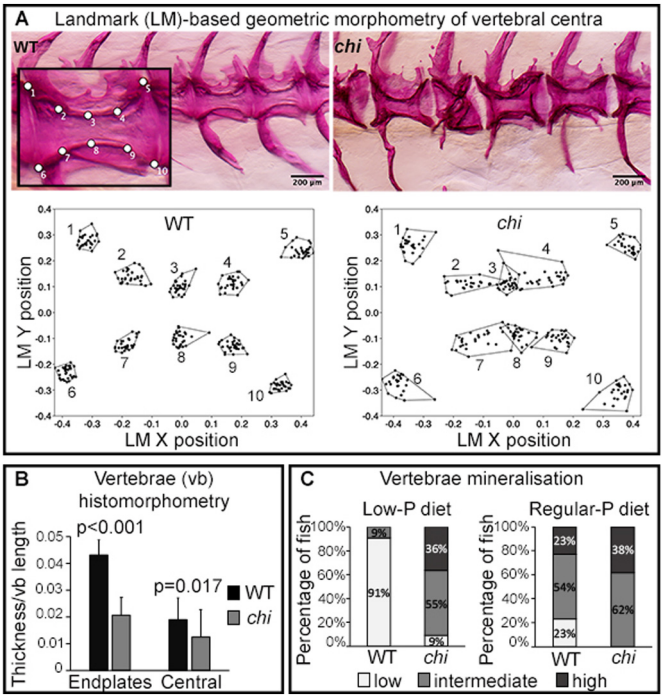
Background/Introduction: The *chihuahua* (*chi*) zebrafish is an established model for human classical osteogenesis imperfecta (OI), a bone disorder caused by mutations in collagen type I. Adult *chi* display hallmarks of human OI, i.e. bone deformities, fragility and high mineral content. The *chi* bone phenotype appears at juvenile stages and worsens with age.

Purpose: Analysis of the early *chi* bone phenotype. Given that low dietary phosphorus (P) intake can increase bone matrix formation and prevent bone mineralisation in wildtype zebrafish (Cotti et al. 2020, Int J Mol Sci, 21, 5429; doi:10.3390/ijms21155429), it was tested whether dietary treatment can reduce excess mineral-to-matrix deposition in *chi*.

Methods: One month old wildtype and *chi* zebrafish were fed with low- or regular-P diets for two months (ethical approval 260/2020-PR) and analysed by whole mount bone staining and histological procedures.

Results: Under regular-P diet, *chi* display higher frequencies of kyphosis ($p=0.002$), lordosis ($p=0.005$) and vertebral body compressions ($p<0.001$) compared to wildtype. Landmark-based geometric morphometry reveals a strong shape variation of *chi* vertebral centra compared to wildtype ($p<0.001$) (Fig.1A). In *chi* the thickness of vertebral body bone structures is reduced ($p<0.001$, $p=0.017$) (Fig.1B). Different from wildtype fish, low-P diet does not reduce the mineralisation of *chi* vertebral bodies (Fig.1C).



Conclusion(s): The *chi* vertebral centra are thin, compressed and highly variable in shape. Sustained low levels of bone matrix formation may account for the high level of mineral-to-matrix ratio, even under low dietary P conditions.

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P222 (ND)

Skeletal variations in wild type medaka: Baseline studies on a biomedical model

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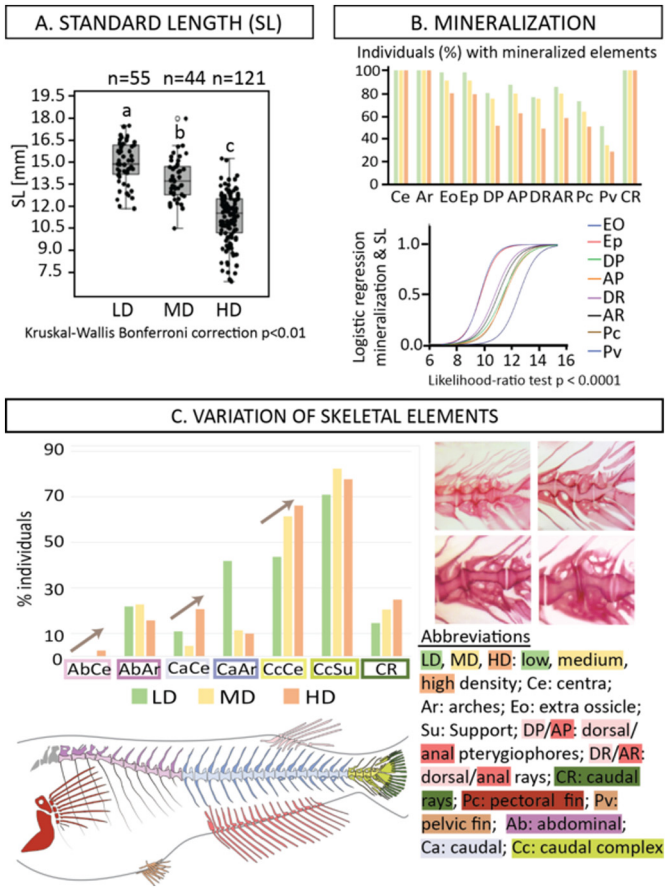
Background/Introduction: *Oryzias latipes* is increasingly used as model for human bone diseases. The variability of skeletal elements under laboratory conditions is an important component of diagnosing mutant phenotypes. Our knowledge about type and extent of skeletal variations in this species is scarce, particularly related to rearing density.

Purpose: Aims are (I) to provide a comprehensive overview of axial skeleton variations in wild type medaka; (II) investigate the

skeletal response to different rearing densities in terms of animal size, number and shape of skeletal elements, mineralisation, and presence of skeletal defects.

Methods: After hatching animals were reared in a recirculating system for 40 days at 3 different densities: low (LD = 5 fishes/L), medium (MD = 15 fishes/L) and high (HD = 45 fishes/L). Ethical approval: 133/2021-PR. The analysis was based on whole mount staining with Alizarin red S, on histological and enzyme histochemical protocols.

Results: HD juveniles had a significantly reduced average length and a wider length distribution than LD or MD animals (Fig.A). A reduced mineralization of skeletal elements in HD animals was correlated with the standard length, indicated by logistic regression analysis (Fig.B). Rearing density had no effect on numbers of vertebral bodies or arches. Vertebral bodies and arches that support the caudal fin are most plastic (Fig.C) concerning shape and fusion of skeletal elements. Vertebral centra and arches are developmental modules, accordingly variations of these elements are uncoupled.



Conclusion(s): A comprehensive axial skeleton overview is provided. Rearing density affects the skeleton of medaka and should be considered when phenotyping. Funding: EU-H2020-MSCA-766347.

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P223 (ND)

Are non-fracture vertebral deformities more prevalent in patients with osteoporotic vertebral fractures?

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Background/Introduction: Non-fracture vertebral deformities (NFDs) are common anatomical variants of the spine. They can alter the biomechanical loading of the vertebrae and may increase vertebral fracture prevalence.

Purpose: We aimed to (i) calculate the prevalence of NFDs in a patient population and (ii) ascertain whether the presence and type of NFD is associated with osteoporotic vertebral fractures (VFs).

Methods: Patients (age = 71.4 ± 11.2 years, 336 women, 134 men) with (n = 250) and without (n = 250) prevalent VFs were studied. Patients had undergone DXA and VFA (Hologic) and additional spinal imaging, if indicated, as part of their Fracture Risk Assessment (FRAS) pathway referral. An expert reader with access to VFA images only (ER) and a musculoskeletal radiologist with access to all spinal imaging (MR) used the Algorithm-Based Qualitative (ABQ) approach to identify prevalent VFs. ER also identified and characterised common NFDs using ABQ.

Agreement (Kappa (κ)) between ER and MR for VF was calculated. Relationships between NFDs and prevalent VFs were examined using Chi-squared testing (P<0.05).

Results: Per-patient agreement for VF was excellent (κ=0.940). Per-vertebra agreement ranged from κ=0.541 at T5 to κ=0.958 at L2.

Table 1
Prevalence of NFDs and relationship to prevalent VFs.

NFD Type	Prevalence in NFD group (n (%))	Prevalence in VF group (n (%))	Relationship to VFs (p value)	Relationship Type
Any NFD	212 (100)	90 (36.0)	0.004	Inverse
Osteophytes	88 (41.5)	36 (14.4)	0.06	-
Degenerative Changes	68 (32.1)	37 (14.8)	0.4	-
Schmorl's Nodes	30 (41.2)	10 (4.0)	0.06	-
Short Vertebral Height	15 (7.1)	5 (2.0)	0.2	-
Cupid's Bow	6 (2.8)	2 (0.1)	0.4	-
Scheuermann's Disease	5 (2.4)	0 (0)	0.03	Inverse

Conclusion(s): Although both VFs and NFDs occurred more frequently at the thoracolumbar junction, NFDs were inversely related to VFs. Thus, it is unlikely that NFD are an important cause of VF.

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P224 (ND)

Identification of modifier genes underlying intra-familial phenotypic variability in zebrafish OI models using whole exome sequencing (WES) and linkage analysis

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