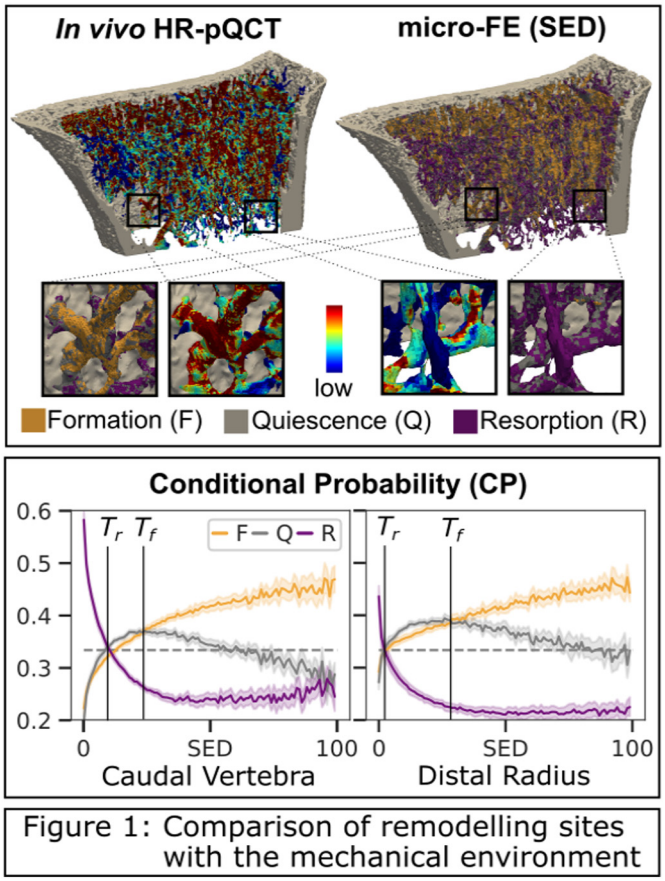

Figure 1: Comparison of remodelling sites with the mechanical environment

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P206 (ND)

Identification of skeletal deformities towards deep phenotyping of zebrafish (Danio rerio) connective tissue disease models

Caitlin Debaene, Adelbert De Clercq, Andy Willaert, Paul Coucke
Ghent University, Biomolecular Medicine, Ghent, Belgium

Background/Introduction: Skeletal deformities in teleost fish have already been extensively described in studies on Atlantic salmon and zebrafish. Nevertheless, a toolset encompassing thorough identification and description of vertebral column deformities to study zebrafish models for human diseases with skeletal involvement is currently lacking.

Purpose: A detailed characterization of skeletal deformities by identifying, describing and quantifying the anomalies will facilitate the development of a reliable deep-phenotyping tool. This tool can be used to establish data matrices by scoring anomalies present in different zebrafish models, which can be used to quantitatively distinguish mild and severe phenotypes. The ability to determine phenotypic severity in disease models is extremely valuable for proper translation towards human diseases, but also to reveal candidate modifier genes that contribute to intrafamilial skeletal variability.

Methods: Zebrafish, 13 *col1a1a*$^{mh13/+}$, 13 *col1a1a*$^{dc124/+}$, 11 *col1a2*$^{mh15/+}$ and 27 WT siblings, were fixed, and made translucent with a mixture of 4% formalin, Triton X-100 and potassium hydroxide (KOH). Subsequently, whole mount bone staining was performed with an Alizarin red S/KOH solution, followed by clearing in a glycerol series. Observations of the skeleton were made using a binocular microscope (Leica M165FC) with a fluorescent unit and equipped with a Leica DFC 450 C camera.

Results: In total, 15 skeletal deformity types were identified and defined: (i) fusion, (ii) compression, (iii) vertical shift of the vertebra, (iv) fractures, (v) curvy ribs), (vi) extra intramembranous bone on the arches and spines (associated elements)and vertebral centra, (vii) bent associated elements, (viii) double associated elements, (ix) detached associated elements, (x) notochord tissue mineralization, (xi) intervertebral ligament mineralization, (xii) lordosis, (xii) kyphosis, (xiii) scoliosis and (xiv) torsion of the vertebral column around the central axis.

Conclusion(s): Deep phenotyping of zebrafish models for skeletal disease will lead to better understanding of expressed phenotypes and of the underlying mechanisms and may lead to identifying new therapeutic targets.

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P207 (ND)

Another player in the game: BMP signaling directly activates chordoblasts for notochord sheath mineralization and centra growth in zebrafish

Hans-Martin Pogoda, Iris Riedl-Quinertz, Matthias Hammerschmidt
Universität zu Köln, Biology/Zoology/Developmental Biology, Köln, Germany

Background/Introduction: Segmented domains within the notochord epithelium express Ectonucleoside Triphosphate Diphosphohydrolase 5a (ENTPD5a) to allow for an iterative mineralization of the collagenous notochord sheath and thus chorda-centra formation, in zebrafish larvae. Thus, the segmented anlage of the developing spine is laid out. Although Notch and Retinoic acid (RA) signaling are already known to feed into this process, the overall molecular machinery controlling the precisely patterned notochord sheath-ossification remains far from understood.

Purpose: Identification of additional molecular players with crucial functions in early zebrafish spine formation.

Methods: We used transgenic approaches to either increase or abrogate BMP activity, both globally and specifically in chordoblasts, the cell type that constitutes the notochord epithelium. We further applied transgene-mediated ablation of chordoblasts, as well as RA treatments. Phenotypes were assessed by reporter-transgene expression, TEM, immunohiostochemistry, and by *in-vivo* of labeling of mineralized matrix.

Results: We found BMP signaling to be sufficient and required for regulation of *entpd5a* activity within the chordoblast layer, subsequently causing block-centra formation or complete loss of mineralization along the notochord, respectively. Furthermore, sustained abrogation of BMP activity after centra induction leads to decreased growth of these structures.

Via immunohistochemistry detecting the intracellular BMP-signal transducer pSmad1/5/8, chordoblast ablation in parallel to BMP2b overexpression, and chordoblast-specific activation or inhibition of BMP signaling, we identified the notochord epithelium cells as the direct targets of BMP signaling. We also found that in the absence of BMP activity, RA is unable to induce the previously described hyper-ossification along the notochord.

Conclusion(s): We identified BMP signaling as another crucial and direct regulator of *entpd5a* activity within the chordoblast layer. Furthermore, it appears to act epistatically to RA in this context.

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