High-Throughput Craniofacial Development Phenotyping in Zebrafish using VAST BioImager

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Zebrafish is an emerging vertebrate model organism to study human diseases and toxicology [1]. A variety of genetic tools in zebrafish allow *in vivo* modeling of human genetic diseases and regeneration [2]. Zebrafish larvae also have small size and optical clarity which allows easy microscopic evaluation of physiological changes and morphological phenotypes. Conventional imaging approaches involve manual orientation of each larva and are difficult to automate for genetic and drug screens. Here we report how VAST BioImager [3] was used to automate zebrafish larvae positioning, orientation and image collection for a large numbers of zebrafish larvae. Mutations in mapre2 gene lead to defects in normal craniofacial development in zebrafish larvae [4]. Mutations in human *MAPRE2* are linked to circumferential skin creases Kunze type ("Michelin tire baby") [5]. *MAPRE2* encodes a member of tubulin binding family and is thought to control microtubule dynamics and reorganization [6].

Method

Heterozygous zebrafish 1.4col1a1:egfp were outcrossed with AB strain adults. Zebrafish embryos were injected with 1 nL (6ng morpholino and/or 100pg of human MAPRE2 mRNA) at the 1-4 cell developmental stage. 50-100 embryos were injected for each experimental condition. Embryos were then maintained at 28^0 C in E3 media (0.3 g/L NaCl, 75 mg/L $CaSO_4$, 37.5mg/L $NaHC0_3$, 0.003% methylene blue). 2-4 days post-fertilization (dpf) zebrafish larvae were anesthetized with 0.2 mg/ml tricaine and loaded using VAST BioImager for collection of images (Fig. 1) [7][8].
Dorsal and lateral imaging templates were created each day to facilitate precise rotational orientation. The minimum similarity was set at 0.7. Fluorescence images of ventral view in the head area were collected in a fully automated way using Zeiss Axioscope A1 microscope, 5x Fluar objective, and Axiocam 503 camera. Zen Pro (Zeiss) imaging software was integrated to automatically collect images when directed by VAST BioImager. GFP was excited with 470nm LED module.

Figure 1. VAST BioImager mounted on high-resolution compound microscope for automated positioning and imaging of zebrafish larvae.

Results

Morpholino suppression of mapre2 in zebrafish larvae resulted in craniofacial development phenotypes (Fig. 2A). First, fish injected with morpholinos had significantly increased the angle of the ceratohyal cartilage in 2-4 dpf larvae (Fig. 2B). In addition, test fish had a delay in the formation of ceratobranchial (cb) arch patterning. Most of the control 3dpf fish had three cb arches while less than 10% of morpholino injected fish showed this phenotype (Fig. 2C).

The similar pathophysiological phenotype was consistently observed using different morpholinos designed to mapre2. This craniofacial model was further tested to assess if complementation with human mapre2 mRNA will reverse the phenotype. Cb-directed arch formation in 3dpf was reversed to almost normal morphological levels. Furthermore, fish tested with mutated mapre2 mRNAs (missense mutations occurring in individual patients) was significantly worse that complementation with wild-type human MAPRE2.

Conclusions

VAST BioImager allowed for the development of a method and testing of a model for high-throughput phenotypic characterization of zebrafish larvae.

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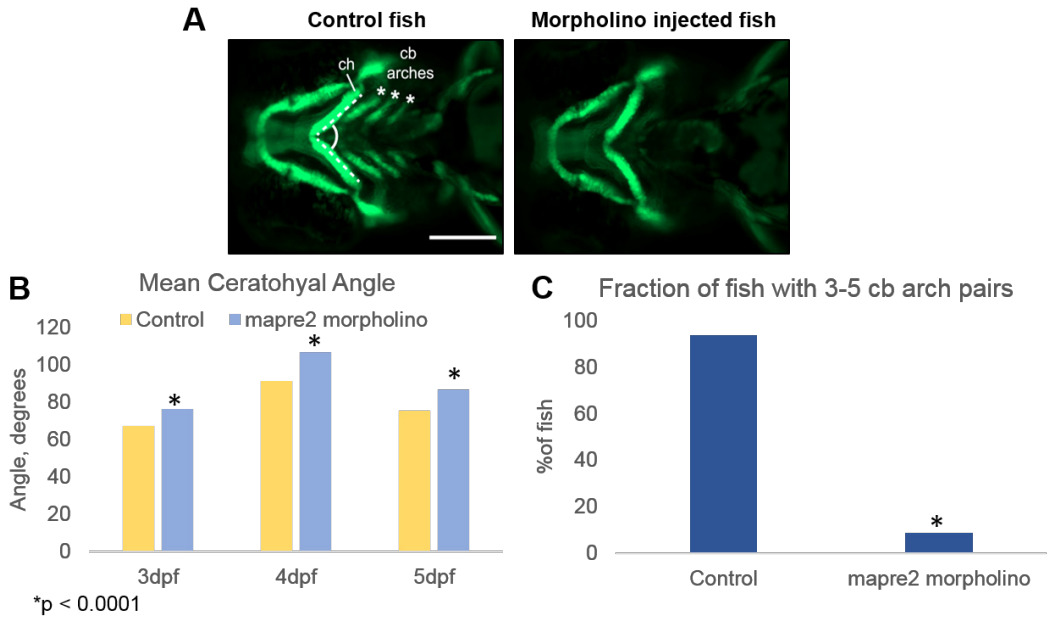

Figure 2. Automated analysis of zebrafish larvae using VAST BioImager. Fish injected with mapre2 morpholinos demonstrate changes in craniofacial development. A. Phenotype of fish injected with *mapre2* morpholinos was quantified using ceratohyal (ch) angle and fraction of fish with 3-5 ceratobranchial (cb) arches. Scale bar, 200 µm. B. Cb angle measured using high-resolution fluorescence images of fish positioned with VAST BioImager. Each bar graph represents mean of 20-48 larvae. C. Fraction of zebrafish larvae with 3-5 cb arches. *Mapre2* depleted fish had delay in cb arches formation compare to wt control. Images of 36–48 fish were collected for each condition using VAST BioImager automation and Zeiss compound microscope. Figure is adapted from [4].

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