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Combined Antiresorptive and Anabolic Drug Approach in Osteogenesis Imperfecta Zebrafish Models: A Geometric Morphometrics and Shape Analysis Perspective

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INTRODUCTION

Osteogenesis imperfecta (OI) is a heritable connective tissue disorder primarily affecting type I collagen biosynthesis, leading to brittle bones and skeletal deformities. Current pharmacological strategies provide only partial therapeutic benefits. Recent preclinical efforts have explored combined antiresorptive and ER-stress modulating therapies. Zebrafish (Danio rerio) models of OI, such as chihuahua (col1a1a mutant) and p3h1 knockout lines, mirror key features of human disease and allow high-throughput skeletal phenotyping. Traditional microCT parameters, while useful, fail to capture spatial morphological changes. Geometric morphometrics (GM) enables the quantification of shape variation, offering higher sensitivity in detecting treatment effects and genotype-specific phenotypes [1]. Analyzing biological shape under experimental conditions requires statistical tools that go beyond traditional size or volume measurements.

OBJECTIVES

To develop and test a reproducible statistical pipeline for assessing shape variation and treatment effects in OI zebrafish models using 2D landmark data, with a focus on the methodological robustness of Procrustes ANOVA under small-sample conditions and the use of simulated data for model validation.

METHODS

The study used vertebral landmark data (10 2D landmarks per vertebra, three vertebrae per individual) collected from wild-type and mutant zebrafish (col1a1a and p3h1), each exposed to four treatments (control, 4PBA, ALN, combined). We performed Generalized Procrustes Analysis (GPA) to align configurations and remove non-shape variation. To assess treatment and genotype effects, we fitted Procrustes linear models (procD.lm) with interaction terms and permutation-based ANOVA (n=3000) [2,3]. The small sample size was addressed through simulation of shape data using multivariate normal distributions based on the empirical mean and covariance structure for each group [4]. Additionally, Partial Least Squares (PLS) was used to investigate covariation between shape and treatment conditions [5]. PCA was used for visualization of simulated vs observed data in tangent space. Differences between real and simulated data were evaluated visually, verifying that shape variance was adequately captured.

EXPECTED RESULTS

We anticipate that genotype will significantly influence vertebral shape across all types, consistent with known skeletal phenotypes in *col1a1a* and *p3h1* mutants. Treatment effects are expected to be more localized, with the combined antiresorptive and chaperone therapy potentially producing intermediate or synergistic changes in specific vertebral re-

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gions. Simulated datasets will allow us to confirm the statistical stability of Procrustes-based models under small-sample conditions, reducing the risk of false negatives and supporting experimental design optimization.

CONCLUSIONS

This work illustrates a flexible and statistically sound framework to analyze shape data in biological models with limited sample sizes. Simulation of landmark configurations allowed us to confirm inference robustness and guide experimental design. Geometric morphometrics, when paired with Procrustes ANOVA and multivariate simulation, can detect subtle effects and validate assumptions, providing a methodological advance for studies in morphometrics, imaging, and developmental biology.

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