Knockout of Dnd1 Reveals Its Roles in Germ Cells, Sex Development, Fatty Acids Metabolism, and Gut Microbiota in Freshwater Angelfish

Wai-Kwan Chu[1], Shih-Chin Huang[1], Ching-Fong Chang[1,2], Jen-Leih Wu[3], and Hong-Yi Gong*†[1,2]

[1]Department of Aquaculture, National Taiwan Ocean University – Taiwan
[2]Center of Excellence for the Oceans, National Taiwan Ocean University – Taiwan
[3]College of Life Sciences, National Taiwan Ocean University – Taiwan

Abstract

Using CRISPR/Cas9 genome editing technology, we achieved targeted mutagenesis of the *dnd1* gene in freshwater angelfish (*Pterophyllum scalare*). Subsequent analysis revealed that homozygous mutation of *dnd1* resulted in infertility among adult angelfish, characterized by the presence of empty gonad somatic structures without any germ cells. Notably, our observations unveiled a significant male-biased trend in sex development of infertile freshwater angelfish, with the majority exhibiting male secondary sexual characteristics and engaging in normal courtship behaviors. Further investigation indicated a marked upregulation of *amh*, a key marker for Sertoli cells, in the empty gonad structure of *dnd1* KO angelfish, suggesting its pivotal role in male sexual fate determination. Additionally, infertility correlated with a conspicuous increase in visceral fat accumulation, highlighting a potential link between adipose tissue dynamics and reproductive dysfunction. Our research highlights the crucial role of the *dnd1* gene in regulating fertility and feminization in freshwater angelfish. Compared to females, infertile male angelfish exhibited notable changes in gut microbiome diversity, but their overall microbial composition closely resembled that of normal males, suggesting that physiological traits remain male-like despite the loss of reproductive function. Core microbiome analysis identified *Cetobacterium somerae* as the dominant bacterial species, a microbe associated with gut health and antiviral immunity of teleost, in both normal male and infertile male angelfish. Our findings suggest a complex relationship between energy allocation, visceral fat deposition, gonad development and gut microbiota during reproduction in freshwater angelfish.

Keywords: freshwater angelfish, genome editing, dnd1, germ cells, sex development, fatty acids metabolism, gut microbiota

*Speaker
†Corresponding author: hygong@mail.ntou.edu.tw