PPARγ regulates fabp4 expression to increase DHA content in golden pompano (*Trachinotus ovatus*) hepatocytes

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1. Introduction

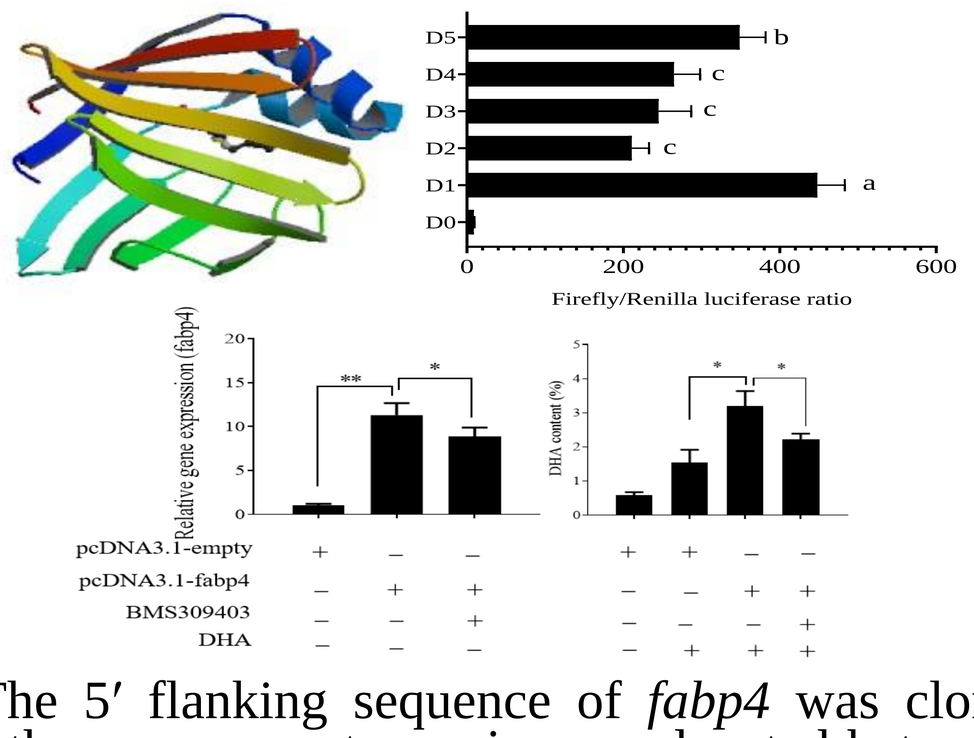
Fish as a main source of n-3 LC-PUFA for human consumers, the n-3 LC-PUFA content of farmed fish is important. In mammals, fatty acid-binding proteins (FABPs) have been reported to be directly related to the fatty acids content. Previously, we identified *fabp4* as a candidate gene regulating n-3 LC-PUFA content by transcriptome analysis. Here, the potential regulatory role and mechanism of *Trachinotus ovatus fabp4* on the n-3 LC-PUFA content were validated.

2. Experimental

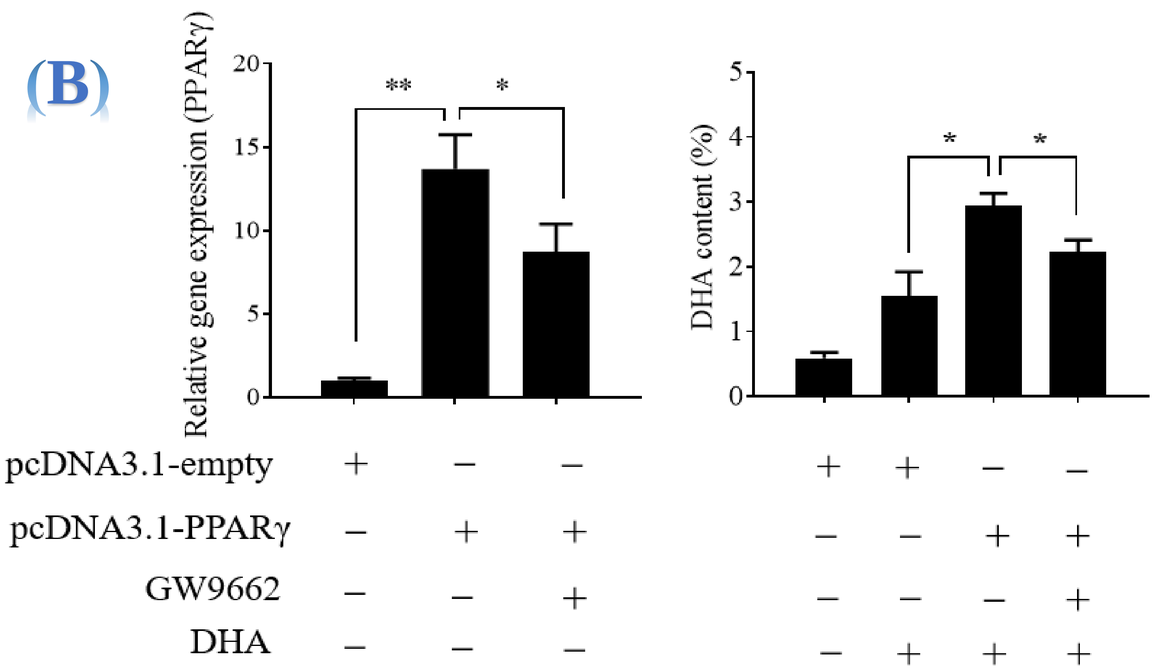
Trachinotus ovatus hepatocyte line used in this study was established previously in our laboratory without publication. Genome walking, quantitative real-time PCR, fatty acid composition analysis, gene overexpression, site-directed mutagenesis, and dual luciferase reporter assay were performed in this experiment to validate and characterize the potential regulatory role and mechanism of *T. ovatus fabp4* on the n-3 LC-PUFA conten.

3. Results

(A)



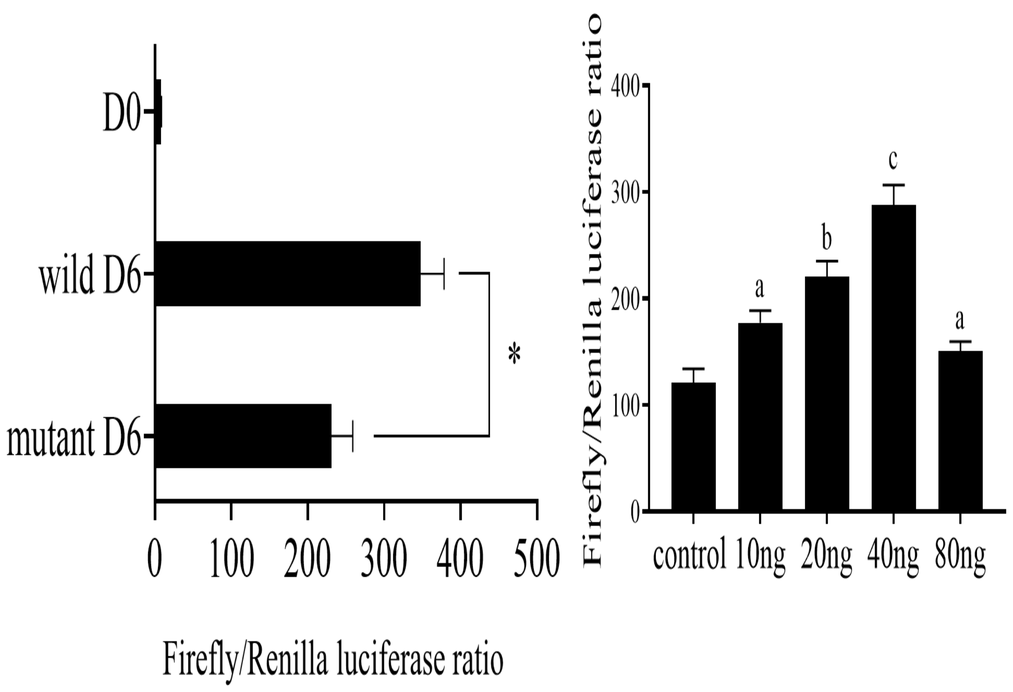
(B)



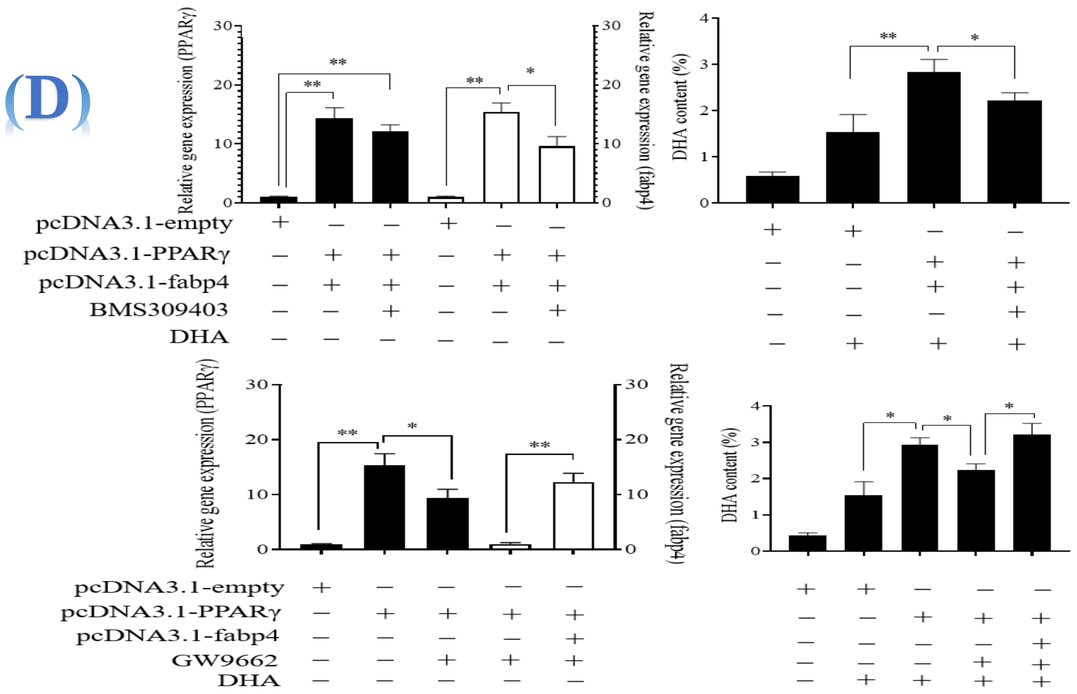
☐ The 5′ flanking sequence of *fabp4* was cloned, and the core promoter region was located between -2006 bp and -1521 bp. The DHA content increased significantly in the *fabp4* overexpression group, and the content of DHA was decreased after the *fabp4* gene was suppressed by inhibitor of *fabp4*, BMS309403.

☐ The uptake of DHA in *T. ovatus* hepatocytes was markedly enhanced after *PPARγ* was up-regulated by overexpression. On the contrary, an antagonist of *PPARγ*, GW9662 attenuated an increase in the DHA content by inducing *PPARγ* overexpression., indicating that *PPARγ* contributed to the uptake of DHA.

(C)



(D)



☐ The mutation of the *PPARγ* binding site resulted in a significant decrease in luciferase activity. Co-transfecting HEK 293T cells with different concentrations of pcDNA3.1-PPARγ was found to increase the luciferase activity in a dose-dependent manner compared to the control group.

☐ The simultaneous overexpression of *PPARγ* and *fabp4* markedly enhanced the DHA content. However, suppression of *fabp4* attenuated the increase in the DHA content. Besides, an inhibitor of *PPARγ*, GW9662, diminished the increasing of DHA content induced by *fabp4*.

4. Conclusion

➢In the present study, we cloned the 5′ flanking sequence of *T. ovatus fabp4*. Targeting *fabp4*, present an effective strategy for the regulation of the n-3 LC-PUFA content in *T. ovatus* . Our findings also indicated that *fabp4*-mediated n-3 LC-PUFA uptake and deposition are probably regulated by *PPARγ* in *T. ovatus*.