# GENETICS DRIVES HEPATIC TRANSCRIPTOME AND DNA METHYLOME OF FARMED GILTHEAD SEA BREAM AFTER BROODSTOCK NUTRITIONAL PROGRAMMING

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## Introduction

Broodstock nutritional programming improves the offspring utilization of plant-based diets in gilthead sea bream through changes in lipid metabolism. Attention was initially focused on fatty acid desaturase 2 (*fads2*) (the first and rate limiting step in the biosynthesis of n-3 long-chain polyunsaturated fatty acids, LC-PUFA), and selective breeding for enhanced *fads2* expression in broodstock fish improved the offspring utilization of plant-based diets (Xu et al., 2021). Otherwise, *de novo* fatty acid biosynthesis of mono-unsaturated fatty acids offers the possibility to mitigate the signs of deficiencies in n-3 LC-PUFA, and the broodstock nutritional programming with a diet rich in α-linolenic acid served to maintain regulated the enhanced expression of *scd1a* (stearoyl-coenzyme A desaturase) in the gilthead sea bream offspring through changes in DNA-methylation (Perera et al., 2020). How such regulatory processes can be driven by a different genetic background is hardly underlined, and the present study aimed to assess how broodstock nutrition affects differentially the offspring transcriptome and genome-wide DNA methylome of reference and genetically selected fish for growth.

## Material and methods

Gilthead sea bream brood fish belonging to reference (REF) or genetically selected (GS) fish within the PROGENSA® selection program received a diet with low fish oil content during the stimulus phase. Two 5-month old offspring subsets of each genetic background were fed either a control (15% fish meal and 5.7-7.6% fish oil) or a FUTURE (7.5% fish meal and completely devoid of fish oil) diet for about 6 months (challenge phase). At the end of the trial, 6 juvenile fish per each experimental condition were anaesthetized and liver samples were taken for wide-analyses of gene expression (RNA-seq) and DNA methylation, using methyl-CpG-binding domain sequencing (MBD-seq) for a large coverage of the CpG methylome.

## Results and discussion

The offspring of GS fish shared a better performance than those of REF animals during the challenge phase, and differences due to diet (with improved values with the control diet) tended to be lower in GS lineage. Data highlighted a different hepatic transcriptome (RNA-seq) and genome-wide DNA methylation (MBD-seq) pattern depending on the genetic background, which agrees with previous studies in fish (Liu et al., 2022). The number of differentially expressed transcripts (comparing control and FUTURE diets) following the challenge phase varied from 323 in REF fish to 2,009 in GS fish. The number of transcripts of discriminant value by multivariate analysis, and associated enriched functions (Gene Ontology-Biological Process, GO-BP, terms), were also markedly higher in GS fish. Moreover, after selecting differentially methylated (DM) regions with an opposite trend for DNA methylation and gene expression, correlation analysis depicted a hyper-methylated and down-regulated gene expression state in GS fish challenged with the FUTURE diet, whereas the opposite pattern was found in REF fish (Figure 1A). Thus,

the resulting epigenetic clock of the latter animals might represent an older phenotype (Piferrer and Anastasiadi, 2023). Moreover, after filtering for functions with a high representation in GS fish, 115 genes were retrieved as epigenetic markers nutritionally regulated in this group of fish (Figure 1B). Among them, genes within the GO-BP term Lipid metabolic process (23) were the most reactive following ordering by gene expression fold-change, which rendered a final list of 10 top markers with a key role on hepatic lipogenesis and fatty acid metabolism (*cd36*, *pitpna*, *cidea*, *fasn*, *g6pd*, *lipt1*, *scd1a*, *acsbg2*, *acsl14*, *acsbg2*). These top 10 genes also showed a greater concentration of DM CpG sites in the promoter region. Down-regulation of most of those genes agrees with the initial statement that the epigenetic regulation of gene expression due to nutritional programming may preclude an over-expression of specific genes that might result counterproductive in a changing environment.

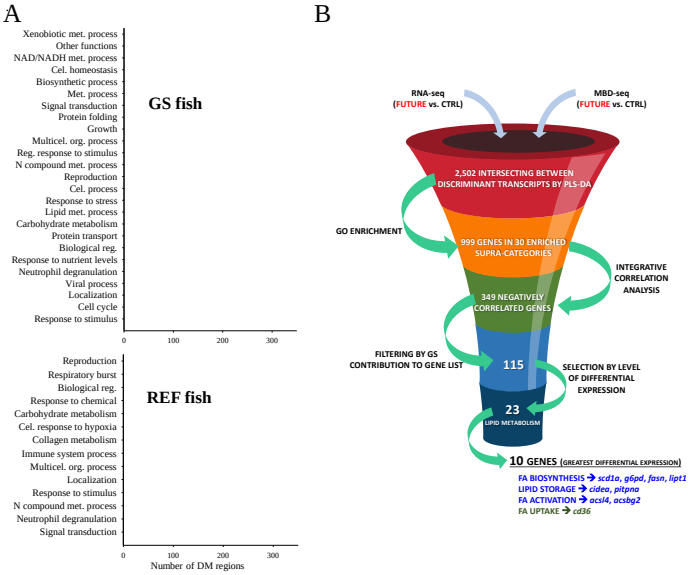


Figure 1. Enriched functions (GO-BP terms) with the number of DM regions showing an opposite trend for methylation and expression in GS and REF fish, being hyper-methylated regions shown in black and hypo-methylated in white (A). Filters applied in data from GS animals to select candidate epigenetic markers of nutritional programming (B).

## Concluding remarks

Gene expression profiles and DNA methylation signatures following nutritional programming were clearly dependent on genetic background in our experimental model. Such assumption affected the magnitude, but also the type and direction of change. Accordingly, the resulting epigenetic clock of REF fish might depict an older phenotype with a lower DNA methylation for the epigenetically responsive genes with a negative methylation-expression pattern. That means that epigenetic markers will be specific of each genetic lineage, serving primarily the broodstock programming in our GS fish to prevent and mitigate later in life the risk of hepatic steatosis due to an exaggerated and/or poorly regulated hepatic lipogenesis when fish facing low fish oil diets.

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## References

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