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IMPACT OF EARLY COLD TEMPERATURE ON GENOMEWIDE DNA METHYLATION PATTERNS IN JUVENILE RAINBOW TROUT DIVERGENT LINES FOR MUSCLE FAT CONTENT.

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Introduction

Recent studies have shown that early exposure to environmental stimuli (such as hypoxia or temperature) could impact fish physiology, growth, metabolism and nutrition at mid- or long term. There are several possible mechanisms underlying this programming phenomenon, among which epigenetic regulations such as DNA methylation (Best et al. 2018). The objective of this study was to understand by which molecular mechanisms early cold exposure (at eyed-eggs stage) can impact fish physiology later in life (at juvenile stage), by analysing DNA methylation patterns in rainbow trout. This study was based on 2 experimental divergent lines for muscle lipid content (Quillet et al. 2005) that have been shown to utilise differently feed and to possess a well differentiated intermediary and energetic metabolism. Our hypothesis is that they will react differently to the cold exposure during incubation.

Materials and methods

At 17 days post fertilization (dpf), eyed-eggs from two experimental lines selected for high or low muscle lipid content, fat line (FL) and lean line (LL), were either incubated at normal temperature (11°C) or incubated at 3°C for 15 days in 12 tanks (2 lines x 2 incubation temperatures x 3 tanks). Hatching and the rest of the rearing was performed at standard temperature (11°C). Eyed eggs were collected at a similar stage of development (220 degree days; 19 and 31 dpf for the control and cold conditions respectively) and analysis of gene expression was performed by qPCR on 5 eggs per line, per incubation temperature and per tank, for 12 genes: *hsp47* (Heat Shock Protein 47), two *cirbp* (cold-inducible RNA-binding protein), *ucp2* (Uncoupling protein 2) and eight *dnmt3* (DNA methyltransferase 3). Genome-wide patterns of DNA methylation was assessed by RRBS (Reduced Representation Bisulfite Sequencing) on liver samples of 48 juvenile trout (2 lines x 2 incubation temperatures x 3 tanks x 4 fish per tank) collected at 189 dpf, snap frozen in liquid nitrogen and kept at -80°C until DNA extraction. Liver was chosen as it is the central organ for intermediary metabolism. RRBS libraries were prepared using MspI and size selection of 40-290 bp fragments and then sequenced on an Illumina NovaSeq6000 sequencer to produce 100 bp paired-end reads (Integrage SA, France). Trimmed reads were aligned to the current Arlee rainbow trout reference genome with the bisulfite mapping tool Bismark. Differential methylation analyses were performed using methylKit (qvalue<0.01, minimal methylation differences between temperature groups>20%). Identified DMCs (Differentially Methylated Cytosines) and DMRs (Differentially Methylated Regions) were finally annotated relative to gene features.

Results

Globally, there were significant but low differences in gene expression for 8 of the 12 targeted genes, with a minor impact of incubation temperature or line at eyed-stage. For the RRBS sequencing data, an average of 47 million paired-end reads were obtained per individual (lowest: 28 million; highest: 74 million). Bisulfite conversion rates were very high (>99.7%). Sequences that aligned to unique positions of the genome represented 50% of all reads on average and were used for subsequent analysis. Numbers of CpGs tested by methylKit (i.e. CpGs with a minimal number of 9 samples in each group satisfying the coverage range of 10-500 reads per CpG) were 1,273,685 for FL line and 1,240,712 for LL line. Differential methylation analyses between control and cold exposure groups revealed 3187 DMCs and 89 DMRs for the FL line; 3442 DMCs and 79 DMRs for the LL line. Annotation of DMCs and DMRs revealed that different genes were involved in the two genetic lines, with a certain degree of overlap (Figure 1).

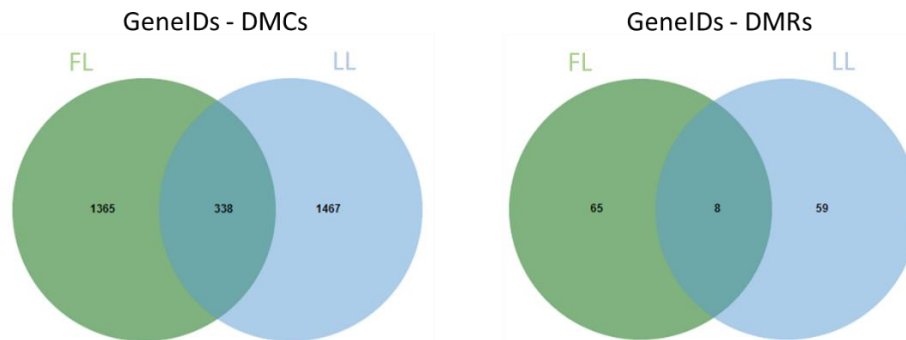


Figure 1. Venn diagrams of geneIDs found in DMCs and DMRs in the two experimental lines, FL (fat line) and LL (lean line).

Discussion

Using two divergent lines for muscle fat content allowed testing the impact of the genetic background on the establishment of DNA methylation patterns in response to early cold exposure. Preliminary results suggest that the two lines responded differently, although some genes with DNA methylation varying according to cold exposure were also found in common. Ongoing interpretation of the biological pathways involved will lead to a finer understanding of underlying mechanisms. This study will contribute to use early programming as a lever to improve long term performances of animals.

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