LONG-LASTING EFFECTS OF TEMPERATURE ON DNA METHYLATION OF MUSCLE AND TESTIS OF THE EUROPEAN SEA BASS (*DICENTRARCHUS LABRAX*)

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Introduction

Temperature during sensitive developmental windows has long-lasting effects on gene expression (Díaz and Piferrer, 2015) and functional processes, such as reproduction and muscle growth (Jonsson and Jonsson, 2014). These effects are mediated, at least partly, at the molecular level by epigenetic mechanisms, such as DNA methylation. Changes of DNA methylation patterns associated with the early developmental temperature a fish experiences have been observed in the gonads at the gene (Navarro-Martín et al., 2011) and at the genome-wide level (Shao et al., 2014; Sun et al., 2016), while in the muscle changes have been observed at least at the gene level (Campos et al., 2013). In this study, we used the European sea bass (*Dicentrarchus labrax*) as a model to study the long-lasting effects of temperature experienced during early development on the methylome and the transcriptome of adult fish.

Materials and methods

European sea bass eggs were obtained from a hatchery and subjected to two distinct thermal treatments from 7 to 68 days-post-fertilization (dpf): maintained at low (16.5-17ºC; LT group) or high (21ºC; HT group) temperature. After that, fish were maintained under natural conditions of temperature until 1122 dpf (~3 years), when testis and muscle were dissected from 3 fish per group. To interrogate the methylome, we used Reduced Representation Bisulfite Sequencing (RRBS). RRBS libraries were prepared, sequenced on Illumina HiSeq 2000 platforms and analyzed bioinformatically. Differentially methylated cytosines (DMC) were identified as the CpGs with more than 15% methylation differences and q-value<0.01. Differentially methylated regions (DMR) were identified by the *edmr* (Li et l., 2013) package.

Results

Differentially methylated CpGs (DMCs) were detected across the whole genome. In the muscle of HT vs. LT fish 17024 hyper-methylated and 16031 hypo-methylated DMCs were detected (33055 DMCs in total). In the testis of HT vs. LT fish 6015 hyper-methylated and 3984 hypo-methylated DMCs were detected (9999 DMCs in total). We, then, focused on differentially methylated regions (DMRs) since these are thought to be crucial for the regulation of gene transcription. In the muscle of FHT fish, 736 DMRs were identified. Six hundred forty eight (88%) DMRs overlapped with CpG islands (CGI) and/or CpG shores (CGS), while 88 (12%) showed no overlap with CGI/CGS. Among the identified DMRs, 638 (87%) overlapped with gene bodies and/or promoters, 98 (13%) were located outside gene bodies and/or promoters, and 180 overlapped with repetitive elements (Fig. 1). In the testis of HT fish, 325 DMRs were detected, among which 297 (91%) overlapped with CGI/CGS and 28 (9%) were located outside CGI/CGS. In gene bodies and/or promoters 208 (87%) DMRs were assigned, while 43 (13%) DMRs showed no overlap with gene bodies and/or promoters. In the testis of HT fish, 44 DMRs overlapped with repetitive elements (Fig. 1).

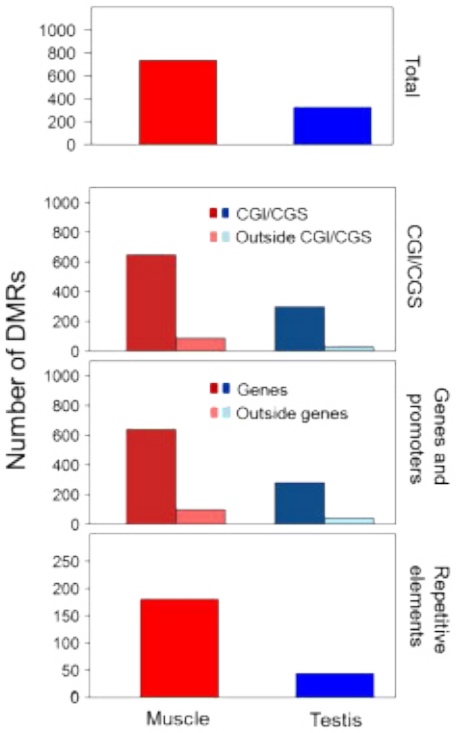

Fig. 1. Number of differentially methylated regions (DMRs) in muscle fish reared at high temperature during early development vs fish reared at low temperature. Numbers of DMRs for each comparison are shown in total; inside CpG islands (CGI) and CpG shores (CGS) and outside CGI/CGS; inside gene bodies and/or promoters and outside gene bodies and/or promoters and inside repetitive elements. Numbers of DMRs in muscle are shown inside genomic features (dark red) and outside genomic features (light red). Numbers of DMRs in testis are shown inside genomic features (dark blue) and outside genomic features (light blue).

Conclusions

Early developmental temperature has long-lasting effects on the DNA methylation patterns of muscle and testis of the European sea bass. These changes occur across the genome and the majority of regions affected overlap with functional genomic elements, indicating possible functional consequences at the gene expression and phenotypic level.

References

Campos, C., Valente, L., Conceição, L., Engrola, S., Fernandes, J., 2013. Temperature affects methylation of the *myogenin* putative promoter, its expression and muscle cellularity in Senegalese sole larvae. Epigenetics 8, 389–397. doi:10.4161/epi.24178.

Díaz, N., Piferrer, F., 2015. Lasting effects of early exposure to temperature on the gonadal transcriptome at the time of sex differentiation in the European sea bass, a fish with mixed genetic and environmental sex determination. BMC Genomics 16. doi:10.1186/s12864-015-1862-0.

Jonsson, B., Jonsson, N., 2014. Early environment influences later performance in fishes: effects of early experiences. J. Fish Biol. 85, 151–188. doi:10.1111/jfb.12432

Li, S., Garrett-Bakelman, F.E., Akalin, A., Zumbo, P., Levine, R., To, B.L., Lewis, I.D., Brown, A.L., D'Andrea, R.J., Melnick, A., others, 2013. An optimized algorithm for detecting and annotating regional differential methylation. BMC Bioinformatics 14, S10.

Navarro-Martín, L., Viñas, J., Ribas, L., Díaz, N., Gutiérrez, A., Di Croce, L., Piferrer, F., 2011. DNA Methylation of the Gonadal Aromatase *cyp19a* Promoter Is Involved in Temperature-Dependent Sex Ratio Shifts in the European Sea Bass. PLoS Genet 7, e1002447. doi:10.1371/journal.pgen.1002447.

Shao, C., Li, Q., Chen, S., Zhang, P., Lian, J., Hu, Q., Sun, B., Jin, L., Liu, S., Wang, Z., Zhao, H., Jin, Z., Liang, Z., Li, Y., Zheng, Q., Zhang, Y., Wang, J., Zhang, G., 2014. Epigenetic modification and inheritance in sexual reversal of fish. Genome Res. 24, 604–615. doi:10.1101/gr.162172.113.

Sun, L.-X., Wang, Y.-Y., Zhao, Y., Wang, H., Li, N., Ji, X.S., 2016. Global DNA Methylation Changes in Nile Tilapia Gonads during High Temperature-Induced Masculinization. PLOS ONE 11, e0158483. doi:10.1371/journal.pone.0158483.