USING TRANSCRIPTOME ANALYSIS TO PROBE THE MECHANISM UNDERLYING INDIVIDUAL VARIATION IN COPING STYLES.

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Introduction

Resolving phenotype variation within a population in response to environmental perturbation is central to understanding biological adaptation. Relating meaningful adaptive changes at the level of the transcriptome requires the identification of processes that have a functional significance for the individual. This remains a major objective toward understanding the complex interactions between environmental demand and an individual's capacity to respond to such demands. The interpretation of such interactions and the significance of biological variation between individuals from the same or different populations remains a difficult and under-addressed question. We previously provided evidence that variation in gene expression between individuals in a zebrafish population could be partially resolved by a priori screening for animal coping styles and accounted for >9% of observed variation in the brain transcriptome (Rey et al 2013). Proactive and reactive individuals within a wild-type population exhibited consistent behavioural responses over time and context that related to underlying differences in regulated gene networks and predicted protein-protein interactions. These differences can be mapped to distinct regions of the brain and provide a foundation toward understanding the coordination of underpinning adaptive molecular events within populations (Rey et al. 2013). In this study, under the COPEWELL EU project, three commercial species (*Salmo salar*, *Dicentratus labrax* and *Sparus aurata*) were used to test for relevant target genes linked to brain expression related to different stress coping styles. Taking a comparative evolutionary approach, zebrafish data on target genes for personality were blasted against salmon, sea bass and sea bream transcriptome resources. Curated lists of mRNA transcripts were used for primer design and validated using pools of fish whole brain cDNAs. Individual absolute quantification of gene expression on four target mRNAs per species is current under analysis. Data on gene expression for each individual will be correlated with the corresponding behavioral data already obtained in coordinated WPs within COPEWELL.
The aim of the TRANSCOPE work package within the COPEWELL framework is to establish methods for reliable identification of contrasting stress coping styles in these species and to provide a causal mechanism for the presence of welfare relevant trait correlations in farmed fish by addressing the genetic regulation of coping styles at the level of the transcriptome.

Materials and Methods

Fish from tanks previously screened for coping styles (Rey et al. 2013, see also Ferrari et al. 2014 abstract on behavioral methods, EAS 2014) were sacrificed with an overdose of Phenoxy ethanol (for seabass and seabream) or MSS-22 for salmon. Brains were extracted and immediately frozen with liquid nitrogen or kept on RNA later at room temperature.
Total RNA was extracted from the brain using TriReagent (Molecular Research Center) following the manufacturer's instructions, and verified for quantity using a NanoDrop ND-1000 (Thermo Scientific) and quality visualized under UV light in a 1% agarose gel containing 1 µg/ml ethidium bromide. 1 µg of total RNA was taken from each individual to synthesize cDNA with SuperScript III RNase Transcriptase (Invitrogen) and oligo-dT primer (Promega). Selected target transcripts were cloned and sequenced.

Conventional PCR (PCR) products were visualised under UV light in a 1% agarose gel containing 1 μg/ml ethidium bromide, purified using PCR clean-up Gel extraction MN (Cultek), cloned into pGEM-T Easy Vector (Promega) by T/A cloning and transfected into competent Escherichia coli DH5αTM Competent Cells, Invitrogen (Promega). Plasmid DNA was isolated by Nucleospin Quickpure (Marcherey Nagel). Absolute Quantification RT-qPCR was performed in a Biometra Thermocycler. Absolute number of copies for each mRNA and each individual were obtained and analysed.
Target mRNAs for *Salmo salar* have been identified and cloned. Of the four mRNAs only one (Alpha 3 ATPase) has been analyzed. Expression analyses for the 3 remaining target mRNAs (IRFN1: Interferon-related developmental regulator 1, PTB: Polypyrimidine tract-binding protein 1 and NEDD8: NEDD8 precursor) is underway.

Statistical Analysis: Tank effects on fish total weight, brain weight and gene copy number were checked with a 1-way ANOVA test and a post-hoc Scheffé test was performed for specific significances. A Two-step cluster for individual gene expression data in each tank was performed with the best number of clusters being 3. Data on copy number was log transformed to normalize. All analysis was performed with SPSS v19 (IBM®). Correlations with behavioral data (Hypoxia test sample 1) were run on AutoDiscovery® (Butler Scientifics) and SPSS v19 (IBM®**).**

Results

Preliminary results on gene expression for Salmon data shows a clear influence of tank on fish Total weight (1wayANOVA; $p<0.000$) differences in mean Total weight between tanks (Post hoc Scheffe test). Also a high correlation between brain weight and total weight was found as expected ($r=0.61$, $p=0.000$) with a similar tank distribution pattern than Total weight. Gene copy number was independent of brain weight (Paired samples correlation, $r=0.015$, $p=0.86$) but there was also a tank effect (One way ANOVA, $p<0.000$; Post-hoc Sheffé test, all statistically different). Based upon these results we decided to analyze each tank independently for mRNA copy number and found 3 preferred clusters for each tank based on individual gene expression (see Fig1). Different percentages of individuals in each cluster size varied depending on the tank where the intermediate group the most over-represented (max 82% to min 54%). No correlations were found between gene copy number and the behavioral variables studied but further data analyses are required.

Discussion and Conclusions

The data obtained allows for a comparative evolutionary comparison of mRNA expression levels in the brain across species correlated to stress coping styles in 4 species of fish. This is a major aim of the TRANSCOPE work package within COPEWELL to explore connections between stress coping style and the underpinning molecular machinery. In this presentation we will discuss the results so far obtained and comment upon them.

References

Rey S, Boltana S, Vargas R, Roher N, Mackenzie S, 2013. Combining animal personalities with transcriptomics resolves individual variation within a wild-type zebrafish population and identifies underpinning molecular differences in brain function. Molec Ecol, 22: 6100-6115.

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