Genome-wide Expression Profiling of Decompression Stress: A Novel Approach to Biomarker Discovery and Validation

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Introduction

Decompression stress (DS) can be defined by the probability of decompression sickness (DCS) as predicted by the U.S. Navy linear exponential multi-gas (LEM) model (Gerth 2002). At present, there is no method for testing the adequacy of such a predictor except to observe DCS (a rare event) or by detecting venous bubbles using ultrasound (which correlates poorly with DCS).

A method by which dives could be monitored for DS could lead to safer and more time effective decompression profiles. Determination of a biochemical marker or a panel of markers for DS would elucidate molecular interactions with gas emboli, which could be a starting point for finding adjunctive or non-recompressive therapies for DCS.

Previous studies have shown that creatine phosphokinase, an indicator of tissue damage, has been shown to elevate significantly in subjects diagnosed with DCS (Martin 1972, Shank 2001). Increased clotting activity and evidence of intravascular coagulation has been demonstrated in DCS cases and in asymptomatic divers (Lee 1998, Radziwon 2007). Inflammatory markers are also present at increased levels in cases of DCS (Montcalm-Smith 2003), and following asymptomatic dives with detectable bubbles (Ersson 2003). However, there are currently no validated biomarkers of DS. Additionally, there are no data available describing interactions of physiological pathways in the pathology of DS or DCS.

Genome-wide expression profiling of peripheral blood mononuclear cells has been used for comprehensive exploration of gene patterns or aberrant gene expression associated with cardiovascular disease states and processes (Aziz 2007). We hypothesize that gene expression microarray technology could be used to identify and validate global gene expression patterns characteristic of DS.

Objective

The goal of this study is to measure peripheral blood mononuclear cell (PBMC) gene expression before and after hyperbaric chamber dives

Methods

- DUHS IRB Pro00013467; NSMRL IRB Pro2008.0007
- N= 9 (US Navy-trained divers)
- No diving, flying, exercise 24 hr pre,12 hr post-dive
- 27 dry, normoxic, light exercise dives
- Three dives per subject: 60, 70 and 80 min @ 47 FSW
- Surface exercise control
- Affymetrix GeneChip U133A 2.0 for expression data

Figure 1: Data Collection Timeline

- - Blood draw (PaxGene tubes), Echocardiogram
- - Depth/time/physical activity recorded
- - Echocardiogram
- - Diver Health Survey

Microarray Data Analysis

- 24,500 probes RMA normalized (Bioconductor/R script)
- Principal Components Analysis to investigate potential presence of batch effect (Bioconductor/R script)
- Step-down permutation t-test (10,000 permutations) to determine differentially expressed genes between pre and post-dive, and correct for False Discovery Rate and Family-Wise Error Rate (Bioconductor/R script)

Results

Figure 2: Principal Components Analysis of complete data set to determine microarray data quality. The proximity of all data points demonstrates the absence of batch effect from sampling and processing methods.

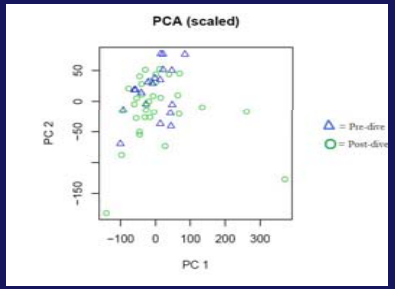
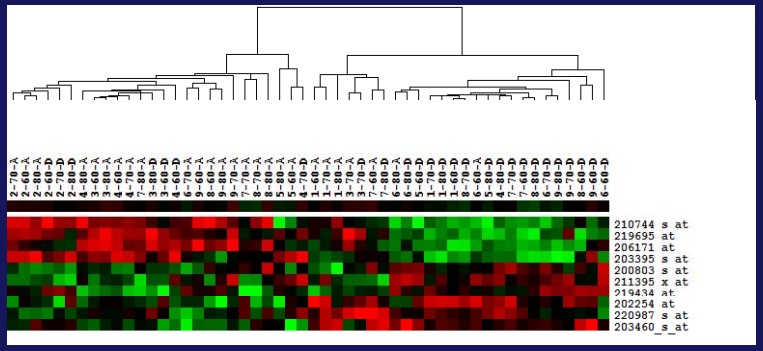


Figure 3: Centroid-linked Hierarchical Cluster Analysis of subject samples based on the top 10 differentially expressed genes, as determined by permutation t-test. Samples are clustered based on similarity of gene expression. Sample ID's (horizontal axis) ending in "A" denote pre-dive samples; "D" denote post-dive samples. Gene probe ID's are on the vertical axis. Red pixels indicate increased expression; green pixels indicate decreased expression.



Discussion

In this preliminary study, we investigated the use of genome-wide microarray to identify global gene expression patterns associated with DS. All dives produced mild to moderate TTE bubble scores (Grade 1-4) in the study participants, except for Subject 3 who had no detectable bubbles during the 70 and 80 minute exposures. Genes differentially expressed at significant levels between pre and post-dive samples include those associated with oxidative stress-activated signaling pathways and immune response (*SOD2, GPX3, ADORA3, SMPD3, IL5RA*). Exercise controls did not exhibit similar gene expression patterns (data not shown). Future studies will further investigate expression of these pathways following high DS dives.

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