IDENTIFICATION OF GENES DIFFERENTIALLY EXPRESSED FOLLOWING NORMOXIC DECOMPRESSION EXPOSURES

Dawn Kernagis*, Michael Datto, David Fothergill† and Richard Moon

Departments of Pathology and Anesthesiology, Duke University Medical Center, Durham, NC

†Naval Submarine Medical Research Laboratory, US Navy Submarine Base, Groton, CT

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 (PBMC) has been used to identify biomarkers and explore gene patterns associated with cardiovascular disease processes (Aziz H. Genom Med 1:105, 2007). This was a preliminary study to test the hypothesis that gene expression microarray technology could be used to identify and validate gene expression patterns characteristic of DS, and potential biomarkers of DS and DCS, by measuring PBMC gene expression before and after hyperbaric chamber dives.

Objective

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

Acknowledgements

The authors acknowledge the Office of Naval Research for funding this research. Special thanks to the staff and volunteer divers at NSMRL for their assistance with this work.

Methods

- DUHS IRB Pro00013467; NSMRL IRB Pro2008.0007
- N= 9 (US Navy-trained divers)
- 27 dry, normoxic dives
- Three dives per subject: 60, 70 and 80 min @ 47 FSW
- Minimum of 1 week between dive exposures
- Light exercise (bicycle ergometer) during the bottom phase
- 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

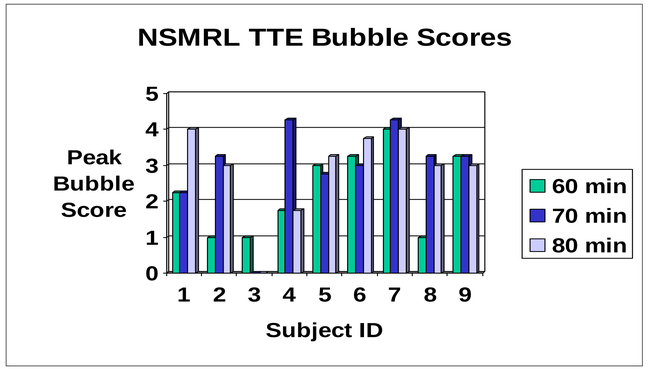

Figure 2: Peak TTE VGE score by subject for each dive exposure (denoted by 60, 70 or 80 minute bottom time)

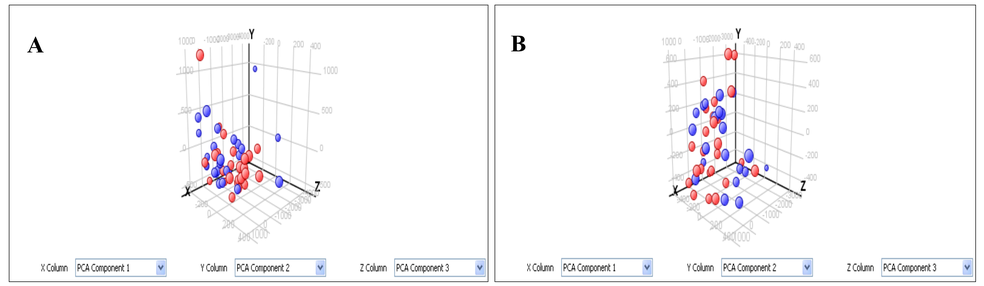

Figure 3: Principal Component Analysis (PCA) on all samples, all probesets A) before QC filter, and B) after QC filter. PCA can be used to detect technical variance due to sample collection or processing. Batch effect appears as distinct separation of samples into clusters, which was not visible in this data set. As seen in 3A, however, there are three outliers that are significantly different from the primary sample cluster. These samples were filtered out and PCA was conducted again to verify lack of batch effect in the remaining samples.

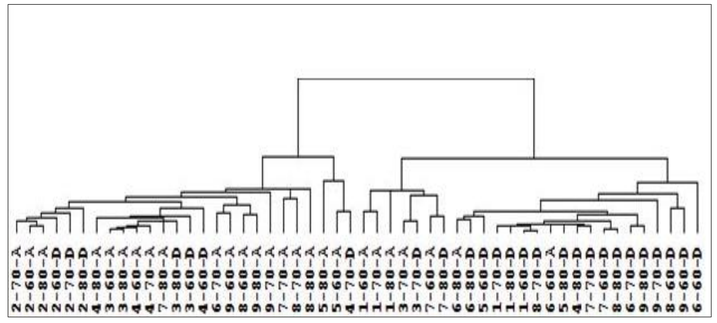

Figure 4: Hierarchical Cluster Analysis tree of all samples based on significant differentially expressed genes. The sample numbers at the bottom of the tree denote "subject ID – bottom time – pre (A) or post (D) dive". Samples are clustered based on similarity of expression. As samples branch closer together on the tree, the similarity in expression among those samples increases.

Discussion

In this preliminary study, we investigated the use of genome-wide microarray to identify global gene expression patterns associated with DS. All dives were asymptomatic and produced mild to moderate venous bubbling in the study participants, except one subject, who had no detectable venous bubbles following the 70 and 80 minute exposures. Comparison of pre versus post-dive gene expression for each individual revealed upregulation of 29 genes and downregulation of 32 genes associated with immune and stress response. Exercise controls demonstrated expression patterns distinct from post-dive samples, with significant changes seen in genes associated with cellular metabolism (data not shown). Gene patterns characteristic of physiological stress following decompression can be identified by genome-wide expression profiling of PBMC's before and after normoxic hyperbaric chamber dives. These preliminary results implicate the involvement of the immune and cellular stress response pathways in response to DS, and demonstrate gene expression profiling as a potential tool to identify biomarkers of DS and DCS in future studies.

References

Aziz H, Zaas A, Ginsburg G. Peripheral blood gene expression profiling for cardiovascular disease assessment. *Genomic Med*. 2007; 1(3-4): 105-112.

Ersson A. Inflammatory response to hyperbaric trauma. An experimental and clinical study. Department of Anaesthesiology and Intensive Care, Malmo University Hospital, Sweden. Doctoral dissertation. 2003 Sep 26.

Gerth WA, Johnson TM. Development and validation of 1.3 ATA PO2-in-He decompression tables for the MK 16 MOD 1 UBA. Panama City (FL): Navy Experimental Diving Unit. Technical Report No.: 02-10. 2002.

Lee HC, Lin TC, Maa HC. Changes in coagulation factor and its correlation with decompression sickness. 1998. Undersea and Hyperbaric Medical Society Meeting Abstract.

Martin KJ. Decompression sickness: Observations on haematological and biochemical parameters. *J Clin Pathol*. 1972 Nov; 25(11):1004-5.

Montcalm-Smith E, Joice R, Porter W, Valentine D, Routh M, Meyerhoff J, et al. Chemokine and cytokine levels and decompression sickness. 2003. Undersea and Hyperbaric Medical Society Meeting Abstract.

Radziwon P, Olszanksi R, Tomaszewski R, Lipska A, Dabrowiecki Z, Korzeniewski K, et al. Decreased levels of PAI-1and alpha$_2$-antiplasmin contribute to enhanced fibrinolytic activity in divers. *Thromb Res*. 2007 May.

Shank E, Muth C. Diver with decompression injury, elevation of serum transaminase levels, and rhabdomyolysis. *Ann Emerg Med*. 2001; 37: 533–6.