

CLASSIFICATION OF GENE EXPRESSION CHANGES INHERENT TO NORMAL BIOLOGICAL VARIANCE VERSUS DECOMPRESSION STRESS EFFECT

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Background

Endpoints of decompression stress are limited

- VGE
- Clinical DCS

DCS pathophysiology is incompletely understood

- Vascular occlusion
- Endothelial dysfunction
 - Levin et al. (1981); Nossum & Brubakk (1999)
- Inflammatory response
 - Ersson et al. (1998, 2002); Bigley et al. (2008)
- Coagulation
 - Boussuges (1998); Olszanski et al. (2001)

Project Hypotheses

- Bubbles can cause decompression sickness by triggering a series of biochemical pathways, which are detectable by blood biomarkers

Table 2 Peripheral blood gene expression profiling in cardiovascular diseases

Indication	Target cell	Results	Ref
Dyslipidemia	Whole blood RNA	895 genes correlated with LDL, 687 with HDL, and 364 with total cholesterol. 188 genes correlated both with LDL and total cholesterol.	(Ma et al. 2007)
Essential hypertension	Whole blood RNA	680 genes were found differentially expressed in untreated hypertensives compared to normotensive controls. On the other hand only 7 genes were differentially expressed in treated normotensives compared to normotensive controls.	(Chon et al. 2004)
Type 2 Diabetes	Whole blood RNA ^a	Among the various pathways analyzed, 48 genes of JNK correlated with diabetes and glycemic control while 92 genes of OXPHOS correlated only with diabetes status irrespective of glycemic control.	(Takamura et al. 2007)
Myocardial infarction	Platelet RNA	From >18,000 probe sets, 54 probe sets showed differential expression between STEMI and stable CAD. Moreover, MRP8/14 predicts risk of future cardiovascular event.	(Healy et al. 2006)
Aortic Aneurysm	Whole blood RNA	A 41 gene signature identified between patients with aortic aneurysm and controls with 78% accuracy along with a sensitivity and specificity of 72% and 90% respectively.	(Wang et al. 2007)
Venous Thrombo-embolism	Whole blood RNA	From 24, 650 genes profiled, 106 gene signature identified (1) patients with APS vs non-APS patients with VTE. (2) Predicted likelihood of APS in patients with VTE. (3) Predicted likelihood of VTE in patients with aPLAs but not APS.	(Potti et al. 2006)
Cardiac transplant	Whole blood RNA	From 252 genes profiled, an 11 gene test discriminated between moderate/severe rejection and quiescence.	(Deng et al. 2006)
Pulmonary hypertension	Whole blood RNA ^a	A 106 gene signature identified with 95–100% accuracy patient with pulmonary hypertension versus controls.	(Bull et al. 2004)

^a Used gradient centrifugation to separate leukocyte fraction

Project Hypotheses

- Bubbles can cause decompression sickness by triggering a series of biochemical pathways, which are detectable by blood biomarkers
- Biochemical mechanisms of DCS can be elucidated by measuring gene expression in circulating blood mononuclear cells

Project Goal

Measure changes in peripheral blood mononuclear cell gene expression and serum markers of tissue injury after high decompression stress dives

Technical Approach/Methodology

ONR BAA# 07-016

“Investigation of Creatine Phosphokinase and Neurological Analytes as Biochemical Markers of Decompression Stress”

Data sources

- Controlled laboratory
 - NSMRL and NEDU

Methods

- NSMRL
- N=9
- 60, 70, 80 min @ 47 FSW
- Dry, normoxic
- Light exercise during bottom phase
- Surface exercise controls



Methods

NSMRL Data Collection Timeline



- - Baseline blood draw, TTE
- - Depth/time/gas profile
- - Blood draw, TTE
- - Post-dive follow-up

Methods

Differential Gene Expression

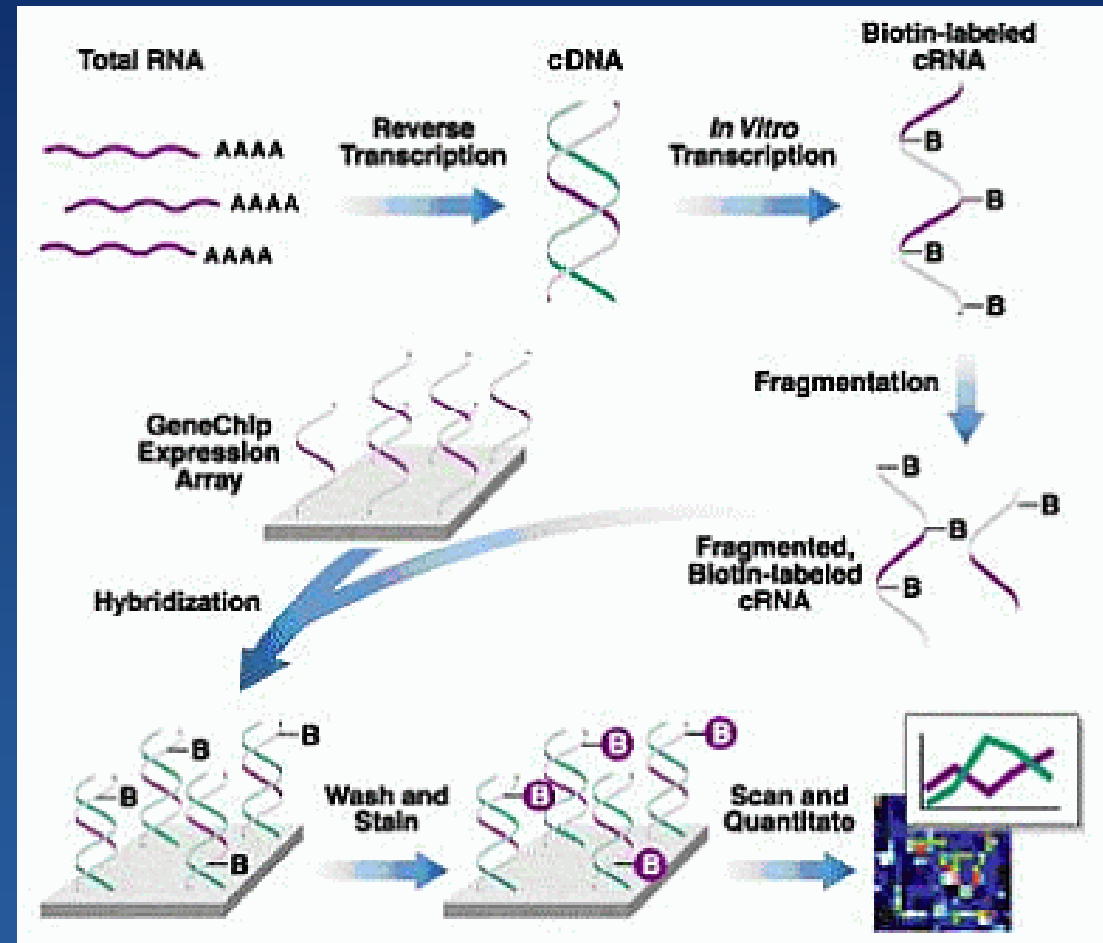
- Comprehensive picture of gene expression pattern
- 8,500 to 38,500 genes

Affymetrix GeneChip®

- Human U133A 2.0
- Expression level of 14,500 well-characterized human genes
- Specific targeting of genes pertinent to endothelial function, inflammation, leukocyte adhesion and coagulation

PBMC

- Monocytes and lymphocytes
- Beta-globin reduction



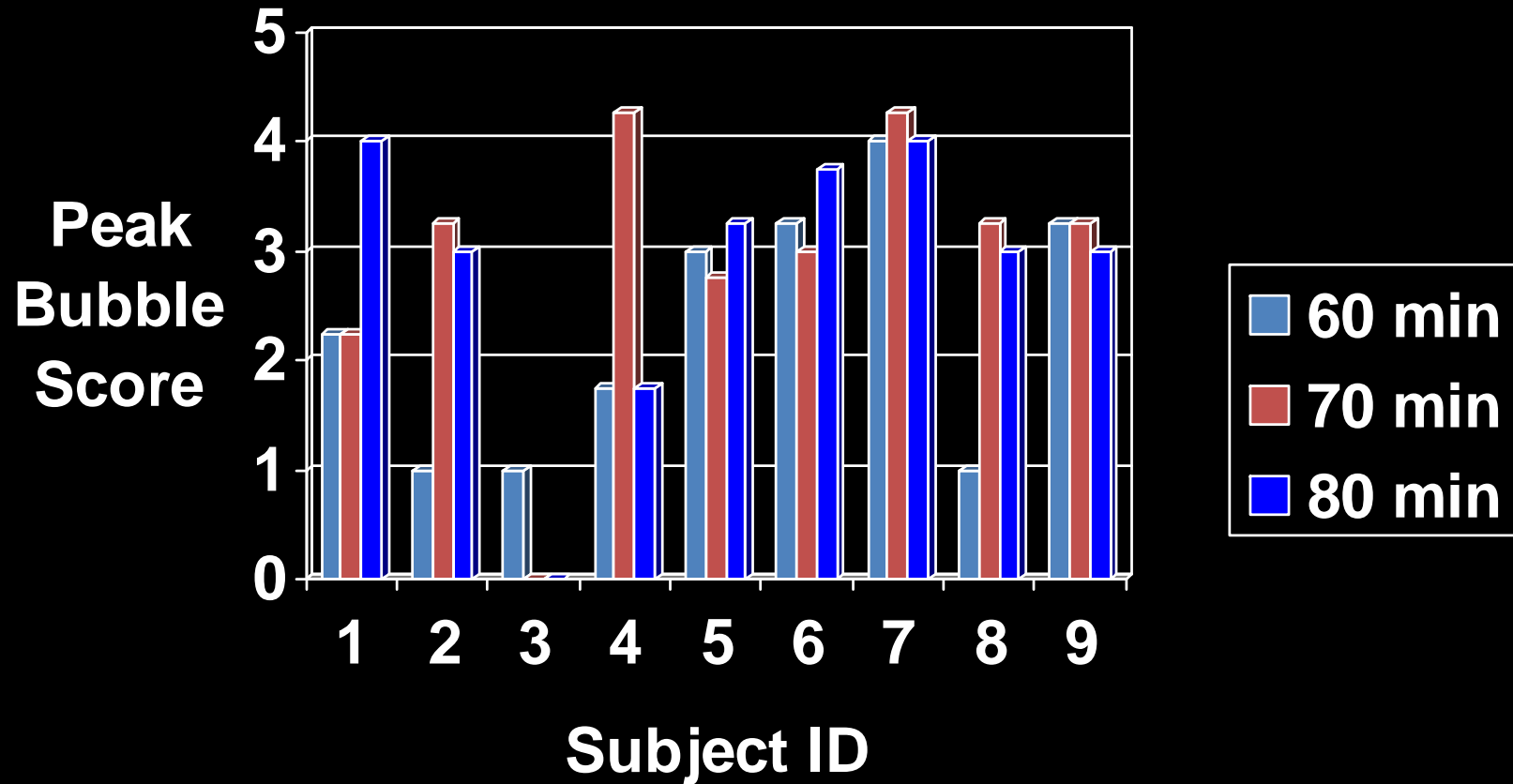
Methods

Microarray Data Analysis

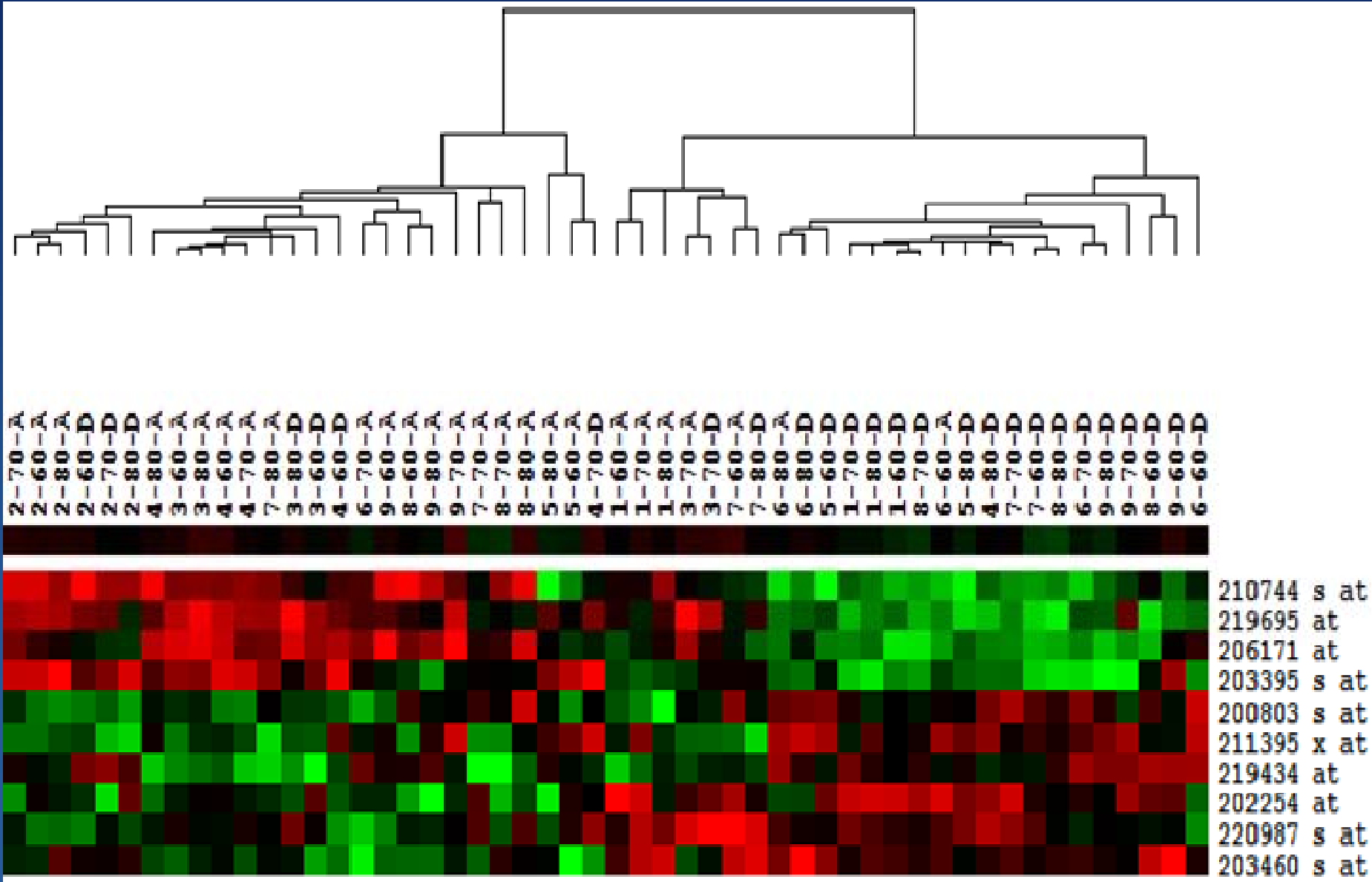
- Statistically significant gene expression
Permutated T-test (corrects for false positives)
- Pattern discovery through Principal Component Analysis, Hierarchical Cluster Analysis
- Biological contextualization
Look at pathways and groups instead of individual genes
- Multiple pattern relationships through methods such as gene and experiment trees

NSMRL Study Results

NSMRL TTE Bubble Scores



NSMRL Results



NEDU and NSMRL Oxygen Control

- 1-hr pre and 2-hr post-dive samples collected as an Annex to NEDU dive trials
- 52 dive profiles with complete pre/post samples; 38 individual subjects
- 29 additional pre-dive samples for normal variance assessment
- NSMRL HBO₂ control samples collected
 - 9 subjects: 1, 2 and 4h @ 2ATA each

Accomplishments

- NSMRL Preliminary data analysis completed
- Completed full study sample collection (NEDU dives and NSMRL HBO study)
- Processed NEDU and NSRML samples for RNA isolation, beta-globin reduction – 176 samples currently undergoing microarray processing!
- Completed 3rd Year of Pre-doctoral studies and Qualifying Examination for Candidacy

Accomplishments



Microarray and Molecular Diagnostics

*Barry WT, ***Kernagis DN**, et al. Intratumor heterogeneity and precision of microarray-based predictors of breast cancer biology and clinical outcome. *J Clin Oncol*. 2010 May 1;28(13):2198-206.

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James ML, et al. A novel inhibitor of neuroinflammation improves outcome from acute brain injury. *Under review*.

Environmental Physiology

Wester TE, et al. Effects of head and body cooling on hemodynamics during immersed prone exercise at 1 ATA. *J Appl Physiol*. 2008 Nov 20.

Peacher DF, et al. Effects of hyperoxia on ventilation and pulmonary hemodynamics during immersed prone exercise at 4.7 ATA: possible implications for immersion pulmonary edema. *J Appl Physiol*. 2010 Jul;109(1):68-78.

Fraser JA, et al. Risk factors for immersion pulmonary edema: hyperoxia does not attenuate pulmonary hypertension associated with cold water immersed prone exercise at 4.7ATA. *J Appl Physiol*. 2010 Dec 9.

Next Steps

- Full study data analysis completion (Summer 2011)
- Publish NEDU and NSMRL study results
- Begin validation study at Duke University

ONR Grant: “Identification of Blood Biomarkers of Decompression Stress through Genome-Wise Expression Profiling”, Start date June 1, 2011

- 60 civilian participants
- 3 profiles each: 3 P(DCS)
- 10 subjects undergo additional HBO control exposure
- Samples for RT-PCR, SNP and serum
- Approved by DUHS IRB (Pro00012945)

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Questions?