Using a Non-supervised Network Analysis to Contextualize a 28 Predictive Gene Classifier Accessing Functional Recovery Potential of Patients Undergoing Mechanical Support

Ryan Togashi*, Galyna Bondar, Eleanor Chang, Tra-Mi Bao, Josephine Hai, Amy Le, Desai Chu, Lindsay Masukawa, Martin Cadeiras, Tristan Grogan, David Elashoff, Maura Rossetti, Elaine Reed and Mario Deng

*This undergraduate worked under the supervision of: Lab Techniques: Galyna Bondar, Ph.D. gbondar@mednet.ucla.edu, Principal Investigator: Mario Deng, M.D. mdeng@mednet.ucla.edu

Background

Of the 300,000 people in the United States who are affected by Advanced Heart Failure, 10% are candidates for Mechanical Circulatory Support (MCS). The leading cause of death for MCS occurs post-surgery with the development of Multiple Organ Dysfunction Syndrome (MOD).

We had previously identified a 28 molecular biomarker test from patients undergoing MCS in a study with patients undergoing MCS-surgery in a tertiary academic medical center between August 2012 – 2014. Twenty-nine patients were included, grouped into two clinically relevant organ failure risk strata: Group I=IMPROVING (SOFA and MELD-XI scores both improve from day -1 to day 8) and Group II= NOT IMPROVING (SOFA and/or MELD-XI score(s) do not improve from day -1 to day 8). Clinical data and PBMC samples were collected one day before surgery (day -1). Samples were processed for PBMC isolation for whole genome mRNA sequencing (Bondar 2017).

Hypothesis

In this study, using non-supervised systems biology strategy, 1) we define the relationship of these genes to PBMC module eigengenes to understand their biological role in organ function recovery and long-term survival, and 2) we hypothesize that other possible transcripts within coexpression network theory may correlate with long-term survival.

Methods

Statistical analysis using Weighted Gene Co-expression Network Analysis (WGCNA) (Rstudio) was used to develop a non-supervised framework to define biology of Differentially Expressed Genes (DEGs). Principal Component Analysis (PCA) was used to reduce dimensionality and identify module eigengenes. Gene Ontology (GO) was applied to interpret the biological representation of the 18 eigengene modules. 11 gene lists were compared across 25 prediction models using Support Vector Machine to predict Group I vs. II membership (**Figure 1**).

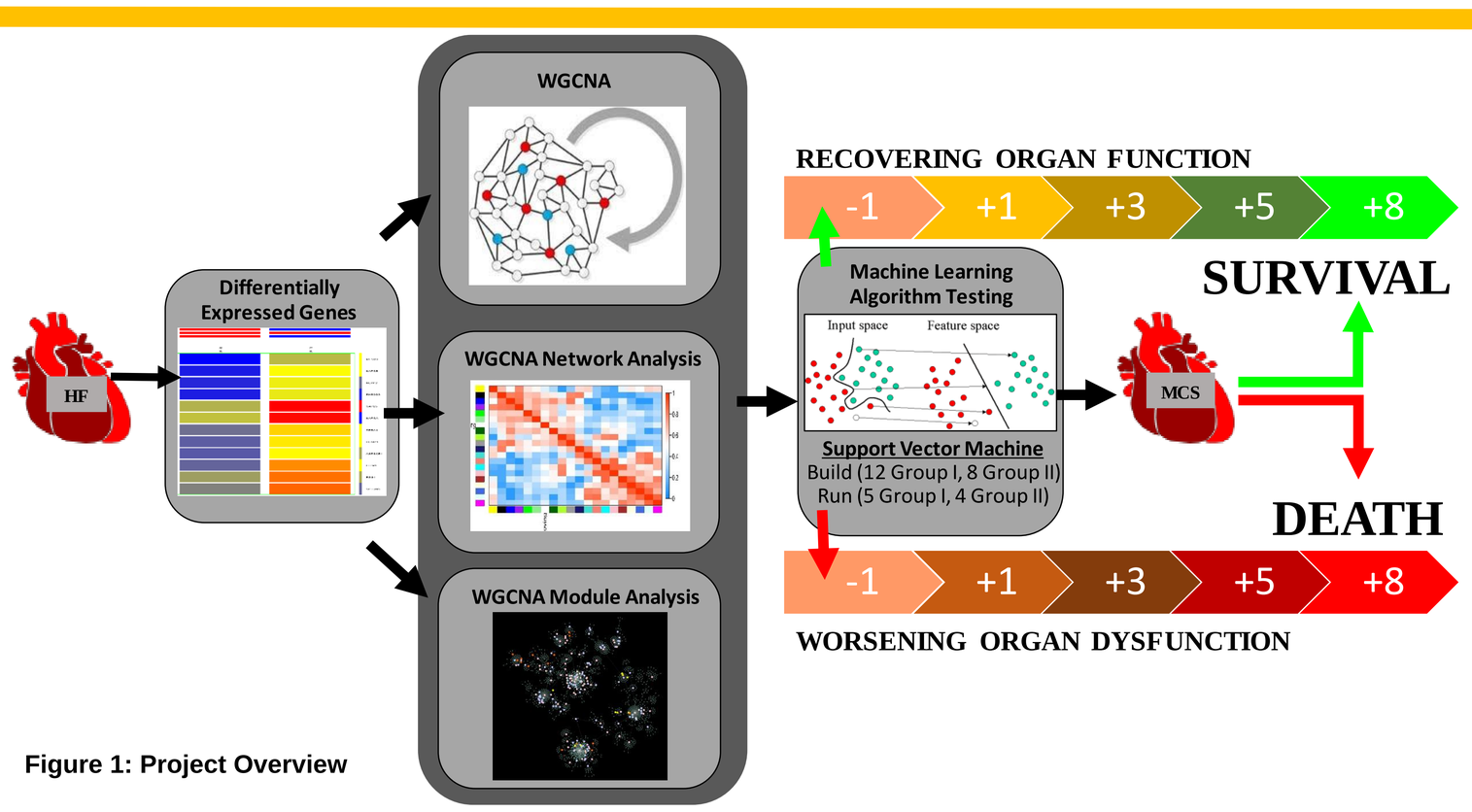
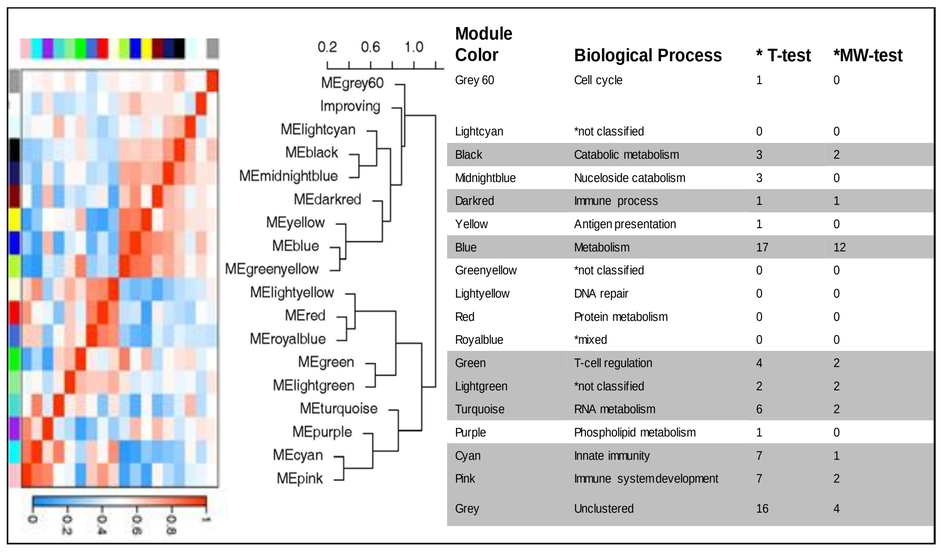


Figure 1: Project Overview

Results

- 19,694 genes were clustered into 18 modules using WGCNA. The clinical trait “Improving” was correlated to eigengene modules. 28 MW and 71 MT DEGs separated into 10 of 18 modules and 13 of 18 modules, respectively. 12 MW and 17 MT genes clustered in the “Blue” module involved in metabolic processes (Figure 2).
- The Blue Module which represents Metabolism contains the most amount of DEGs from both MT (17) and MW (12) genes. Hub genes and their highly connected network properties are thought to be a possible target for risk prediction in coexpression networks. However, DEGs do not fall within any definition of hub genes for the top 1%, 5%, and 10% with the exception of one MT falling in the top 10% (Figure 3).
- MW DEGs remains the most accurate predictor of Organ Function Improvement using support vector machine (Figure 4).
- When conducting whole Network Analysis with all 1% hub genes added to the 28 MW DEGs list, it did not improve accuracy. Hub genes may not be as important as prediction genes and may serve other measure of intramodular network connectivity for module membership as suggested by Yang 2017 (Figure 4).



Module Color	Biological Process	* T-test	*MW-test
Grey 60	Cell cycle	1	0
Lightcyan	*not classified	0	0
Black	Catabolic metabolism	3	2
Midnightblue	Nuceloside catabolism	3	0
Darkred	Immune process	1	1
Yellow	Antigen presentation	1	0
Blue	Metabolism	17	12
Greenyellow	*not classified	0	0
Lightyellow	DNA repair	0	0
Red	Protein metabolism	0	0
Royalblue	*mixed	0	0
Green	T-cell regulation	4	2
Lightgreen	*not classified	2	2
Turquoise	RNA metabolism	6	2
Purple	Phospholipid metabolism	1	0
Cyan	Innate immunity	7	1
Pink	Immune system development	7	2
Grey	Unclustered	16	4

Figure 2: Module-Module Relationship

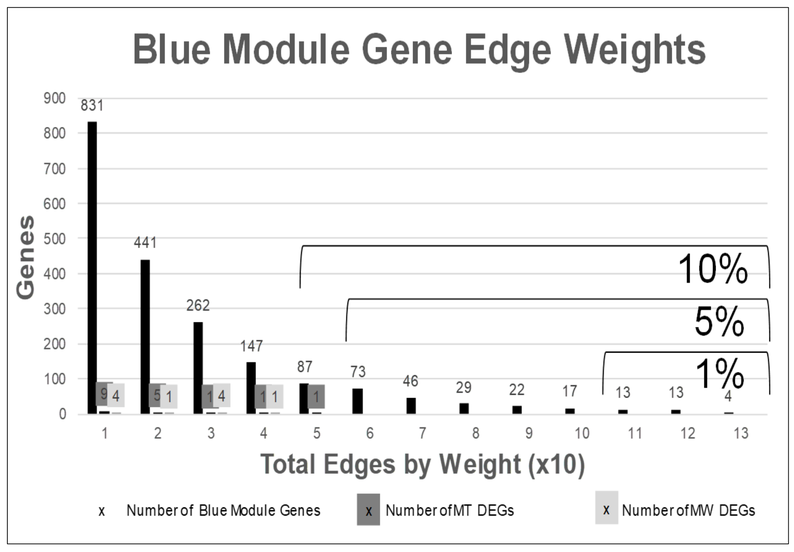


Figure 3: Hub Gene and DEGs

	Blue Module Analysis								Network Analysis					
	DEGS		Hub Genes				Combined		DEGs		Hub Genes		Combined	
	MW	MT	1% Hub	5% Hub	10% Hub	PCA	MW DEGs + 1% Hub		MT	MW	All 1% Hub Genes	9 Modules with DEGs	MW DEGs + All 1% Hub Genes	MW DEGs + 1% Hub (9 Modules)
Number of Genes	12	17	98	98	196	5	31		71	28	150	70	178	98
Averages	57.78	58.67	48.00	37.33	34.22	55.56	73.33		89.33	94.22	32.00	39.11	0.80	88.89
Standard Deviation	14.40	16.78	13.23	21.52	16.00	17.21	14.05		5.86	6.38	13.45	12.88	0.18	8.77
Median	55.56	55.56	44.44	33.33	33.33	55.56	66.67		88.89	100.00	22.22	44.44	44.44	88.89

Figure 4: Whole Network Analysis

Conclusions

Based on our study, leukocyte biology could play an important mechanistic role of understanding MOD. Through the use of a non-supervised co-expression network analysis of 28 MW genes predicting postoperative FRP, we can gain promising insights into the pathophysiology of MOD. Using this approach to generate novel hypotheses, further analyses in larger patient cohorts are necessary to verify the results of the experiment.

Acknowledgements

Funding:
UCLA NIH R21 1R21HL120040-01 (MCD) (PI Deng)
UCLA R01 (PI Weiss, Joint PI Deng)
UCLA R01 (PI Ping, Co-I Deng)
UCLA DOM Internal Funds and the Advanced HF Research Gift to Columbia University (Philip Geier, John Tocco and Robert Milo)
Advanced HF Research Gift to UCLA (Larry Layne, Juan Mulder, and Peter Schultz)

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