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Feasibility and Preliminary Results of Whole Blood RNA-Sequencing Analysis in Patients with Intracranial Aneurysms

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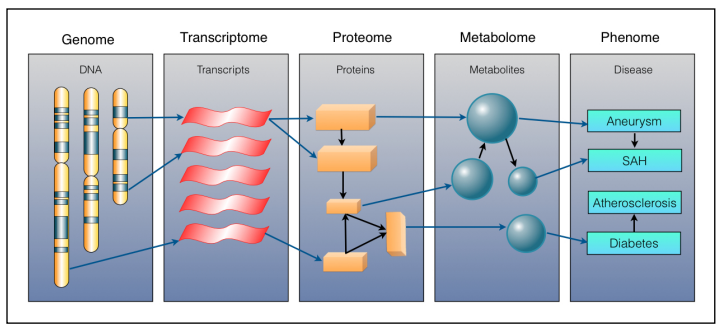
Introduction

Intracranial aneurysms (IA) growth and rupture have been associated with chronic remodeling of the arterial wall. However, the pathobiology of this process remains poorly understood. The objective of the present study was to evaluate the feasibility of analyzing gene expression patterns in peripheral blood of patients with ruptured and unruptured saccular IAs. Transcriptomes reflect the genes that are being actively expressed at any given time, therefore, their study provides a unique window to the dynamic expression level of mRNAs in a given cell population at specific time points. A systematic and throrough evaluation of RNA-sequencing in white blood cells can provide novel avenues to understand some of the potential inflammatory cell-mediated mechanisms impacting the development and rupture of intracranial aneurysms.



Methods

We analyzed human whole blood transcriptomes by performing paired-end, 100 bp RNA-sequencing (RNAseq) using the Illumina platform. We used STAR to align reads to the genome, HTSeq to count reads, and DESeq to normalize counts across samples. Self-reported patient information was used to correct expression values for ancestry, age, and sex. We utilized weighted gene co-expression network analysis (WGCNA) to identify gene expression network modules associated with IA size and rupture. The DAVID tool was employed to search for Gene Ontology enrichment in relevant modules.

Figure 1

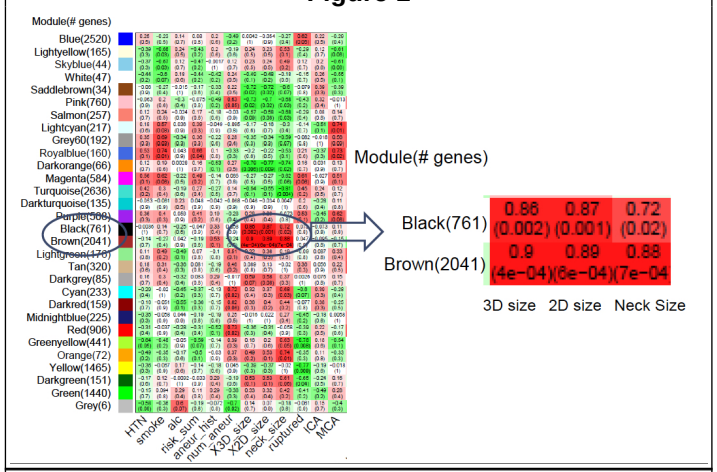
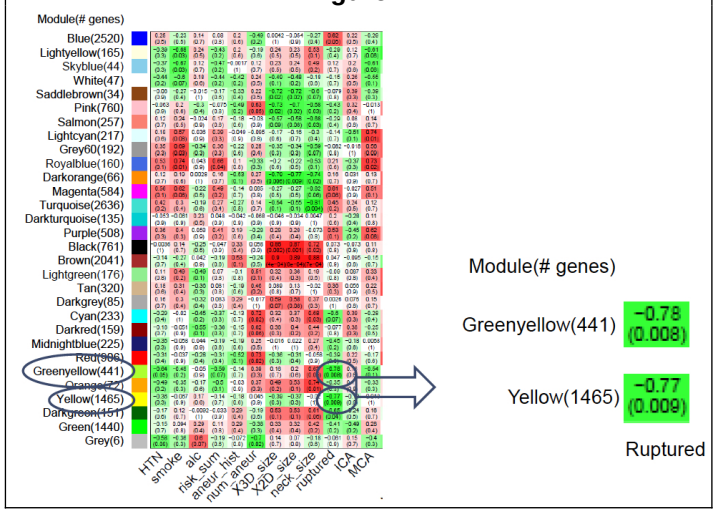


Figure 2



Figures 1 and 2

The rows are labeled by the module eigengene (ME) color. The columns are labeled by the clinical trait. Each square displays the correlation coefficient (r) and the corresponding p value in parenthesis between the ME and each phenotype. Red color represents a positive correlation, while the green color represents a negative correlation.

Results

Samples from 12 patients (9 females, age 57.6 +/- 12) with IAs were analyzed. Four had ruptured aneurysms. RNA isolation and application of the methodology described above was successful in all samples. Although the small sample size prevents us from drawing definite conclusions, we observed promising novel co-expression networks for IAs.

WCGNA analysis showed down-regulation of two transcript modules associated with ruptured IA status (r=-0.78, p=0.008 and r=-0.77, p=0.009, **Figure 1**), and up-regulation of two modules associated with aneurysm size (r=0.86, p=0.002 and r=0.9, p=4e-04, **Figure 2**), respectively. DAVID analyses showed that genes upregulated in an IA size-associated module were enriched with genes involved in cellular respiration and translation, while genes involved in transcription were down-regulated in a module associated with ruptured IAs.

Conclusions

In this study we found that whole blood RNAseq analysis is a feasible tool to capture transcriptome dynamics and potentially achieve a better understanding of the pathophysiology of IAs. Building on these results, we can conduct further longitudinal studies of patients with IAs using network analysis to identify the transcriptome dynamics associated with clinically relevant events such as IA growth and rupture.

References

Villablanca JP, Duckwiler GR, Jahan R, et al. **Natural history of asymptomatic unruptured cerebral aneurysms evaluated at CT angiography: growth and rupture incidence and correlation with epidemiologic risk factors.** *Radiology.* 2013

Langfelder P, Horvath S. **WGCNA: an R package for weighted correlation network analysis.** B*MC Bioinformatics.* 2008