Exploring a Transcriptome-forward Approach for Genetic Evaluation in Thoracic Aortopathy

Katherine Stanley[1], Benjamin Landis[2]

[1]Indiana University School of Medicine; [2]Indiana University School of Medicine, Department of Pediatrics

Background/Objective: Thoracic aortic aneurysm (TAA) is an aortopathy that predisposes to aortic dissection and rupture. Transcriptomic analysis has diagnostic utility in other diseases, but this has not been developed for TAA. We therefore sought to implement the Genome Analysis Toolkit (GATK) as a tool for high-throughput detection of novel variants in RNA sequencing (RNA-seq) data from patients with TAA.

Methods: Human proximal aortic tissue samples from 63 TAA patients and 15 controls were used to establish primary smooth muscle cell (SMC) lines in culture. Total RNA was extracted at early passage; RNA-seq was performed using 150 base pair paired-end reads. Variants were called from RNA-seq reads using the GATK's HaplotypeCaller tool. The MarkDuplicates tool tagged duplicate reads, but these were retained for variant analysis. Singletons were defined as heterozygous variants unique to one sample in the overall group.

Results: The RNA-seq pipeline was validated by confirming known mutations in 2 patients, inspecting reads directly in the Integrative Genomics Viewer (IGV), and genotyping for a common single nucleotide polymorphism. Across all 78 samples, the GATK pipeline identified 4,823,462 variant sites, 236,495 of which were in coding regions. Variant sites were filtered for those located within the coding regions of 31 genes curated for known evidence of aortic disease causality. Among the 511 coding variant sites in these genes, 330 single nucleotide variants and 33 insertions/deletions were identified as singletons. Singleton variant calls were evaluated for quality and annotated in order to predict their likelihood of pathogenicity, thus far identifying strong candidate causal variants in *FBN1*, *TGFBR1*, *TGFB3*, *GATA4*, and *HEY2*.

Conclusion: Application of the GATK RNA-seq pipeline expands our prior IGV analysis, further demonstrating the potential diagnostic utility of transcriptomic analysis of aortic SMCs in TAA.

Scientific Impact: Identifying novel aortopathy-associated variants could improve future disease detection and prediction of severity.