The Development of a Blood Test for Coronary Artery Disease

An academic team employs SeqLL RNAseq to acheive new insights

Tim McCaffrey, Ph.D.

GW and SLI scientists employ SeqLL RNAseq to identify blood biomarkers of disease and guide patient care. The resulting diagnostic tests are being commercialized through True Bearing Diagnostics, LLC.

Tim McCaffrey, Ian Toma, Richard Katz, Jon Reiner, & Ramesh Mazhari.
Divisions of Genomic Medicine and Cardiology, The George Washington University Medical Center, In Partnership with Georges St. Laurent, III, Phillip Kapranov, Dmitry Shtokalo, SeqLL, Inc., and The St. Laurent Institute.

Cardiovascular diseases, particularly atherosclerosis, are the major cause of death and morbidity in developed countries. Atherosclerosis can lead to an acute myocardial infarction (MI), or heart attack, with an incidence of approximately 650,000 per year in the U.S. alone. The current gold standard for diagnosing coronary artery disease (CAD) is coronary artery angiography. Surprisingly, despite some well-established clinical and diagnostic criteria, about 30-40% of the 1 million diagnostic catheterizations each year in the U.S. return a result of 'no coronary blockage'. There is an urgent need for a blood test that will predict the presence of CAD in patients presenting with a clinical suspicion of heart disease.

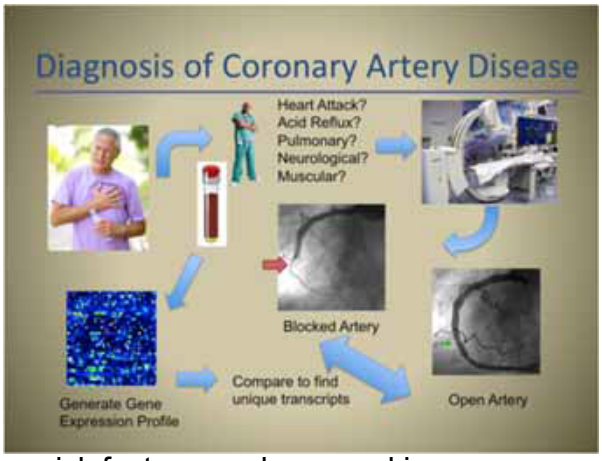
Hypothesis

Patients who develop coronary artery disease produce specific changes in gene expression in whole blood RNA that are detectable by genome-wide RNA sequencing.

Methods

Whole blood RNA was analyzed by the most advanced SeqLL single molecule sequencing (SMS) of RNA (RNAseq, aka 'deep or next-gen sequencing') to identify *tr*anscripts *a*ssociated with *C*AD (TRACs). A development cohort of 112 patients presenting for coronary catheterization was subjected to RNAseq. Blood RNA was depleted of ribosomal RNA and then RNA was sequenced on a SeqLL SMS. The resulting short reads were aligned to the human transcriptome and the number of reads per kilobase of exon per million (RPKM) reads was determined, and then compared between groups by filtering for changes >1.5 fold at a p value < 0.01. A validation cohort of patients will be used to independently verify sensitivity and specificity.

Results



SeqLL RNAseq was highly linear over more than 22 $\log_2$ orders of magnitude of gene expression. About 200 transcripts that differed significantly between groups were identified. The low RNA levels and small magnitude of the changes, less than 2-fold, are uniquely detectable by the SeqLL approach. Further, the low-fold change detection may explain why these transcripts have not been seen in prior microarray-based studies. The TRACs were found to be independent predictors of CAD, essentially unaffected by other known risk factors, such as smoking or hypertension. Careful follow-up analysis and confirmatory studies strongly suggest that these TRACs are RNA markers of a subset of circulating cells, consistent with numerous prior publications suggesting important changes in the immune cell ratios as a contributing factor to CAD.

Significance

These studies potentially provide a clinic-ready diagnostic test for the presence of CAD in chest pain patients. In the future, this test could be expanded toward diagnosing CAD in *asymptomatic* patients, which could potentially prevent unexpected MI and provide physicians the chance for early intervention, with simple, proven therapies such as aspirin, statins, and lifestyle changes.

Project status

The GW/SLI collaboration has completed the first 112 patients on the SeqLL platform. True Bearing Diagnostics is migrating the test into a smaller, faster, cheaper version that examines only the relevant RNA transcripts that were identified. The test will then be validated in a larger, multicenter cohort of >400 patients presenting for angiography or for CT-angiography. The results will provide data for FDA-clearance and a commercial offering of the test as a laboratory developed test (LDT).