Blood and Cardiovascular Disease
The Promise and Limitations of Gene Expression Analysis

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In this issue of *Circulation: Cardiovascular Genomics*, Wingrove and colleagues\(^1\) present data from patients who underwent coronary catheterization and compare a whole-genome microarray from peripheral blood mononuclear cells in case patients (n=27) and control subjects (n=14) without angiographically significant coronary artery disease. Using microarray, they report that >500 genes had more than a 1.3-fold change, with the vast majority being upregulated. Using this information and selecting additional genes reported in the literature, they confirmed changes in gene expression in 2 independent cohorts by reverse transcription–polymerase chain reaction. After multivariable analysis that included some risk factors and a few medications, 14 genes remained significantly associated with cardiovascular disease in 1 cohort and 11 in the second set.

In this study, specific genes were reported as upregulated in patients with angiographically defined significant coronary artery disease in all 3 patient groups. Several genes were confirmed by reverse transcription–polymerase chain reaction and may be related to cardiovascular disease. These included 5-lipoxygenase, which generates leukotrienes and has been associated with atherosclerosis, and, conversely, glutathione-S-transferase, which catalyzes the conversion of lipoxygenase products. The clinical relevance of these observations remains to be defined.

Observations such as those reported by Wingrove and colleagues\(^1\) in this issue of *Circulation: Cardiovascular Genomics* suggest promise for an evolving technology; however, as illustrated by this study, there are many limitations that need to be overcome and issues that must be examined to optimize the utility and reliability of the transcriptomic information. The first is the general concern about the lack of hypothesis-driven experimentation. However, in the present study, genes were chosen for the confirmation sets with an eye toward biological plausibility. In addition, microarray studies by nature are not hypothesis driven. This leads to the second commonly cited concern with high-throughput methodologies, the problem with multiple testing and the false-discovery rate. As with many of the smaller single-nucleotide polymorphism studies, one must be concerned that the information reported in these early gene expression studies may not be confirmed in much larger data sets that will be reported in the near future.

There are also crucial technical considerations that are often ignored but may drive the validity of large gene expression studies in the future. Some stem from the distinct differences between genomic and transcriptomics data. With genomic data, a specific variant is present or not; however, with transcriptomic data, levels will also vary between individuals and in different disease states. There is currently...
no consensus on how those data should be expressed, particularly in larger sets. Unresolved is the issue of whether the use of complementary DNA arrays versus quantitative reverse transcription–polymerase chain reaction provides more reliable determination of messenger RNA levels. Arrays are often normalized to commercially available “normals,” which requires a substantial leap in faith as to what one is controlling for. Quantitative reverse transcription–polymerase chain reaction relies on housekeeping genes, but these genes themselves may be influenced by disease states. A limitation of the present study is that the housekeeping genes chosen (RPL28 and Pro1853) were not validated; only 2 were used, and they have not been shown to be stable in patients with cardiovascular disease.

As discussed, studies that use blood to study gene expression should be larger and more powerful in the future; however, the source of RNA may also drive the results. Whole-blood isolation is simpler than leukocyte isolation and thus is widely used. The assumption of many has been that the source of RNA from the blood is equivalent to any other to detect clinically meaningful differences. This is likely not a valid assumption. It has been shown that whole-blood methods do not provide the same data as isolated leukocytes. Whole-blood sources are significantly less reliable for detecting changes in gene expression in response to stimulation (only one third the number of genes associated with change were detected compared with the number detected with isolated leukocytes), and when whole-blood methods are used, the most predominant messenger RNAs present are residual messenger RNAs associated with erythrocytes or reticulocytes. When the 2 methods are compared, the primary determinant of gene expression is the source of RNA, not the disease state. In addition, a recent large epidemiological study that used isolated peripheral blood lymphocytes demonstrated that common gene variants influence transcript levels and that many are heritable. The source of RNA from the study presented in the current issue of the journal is mixed. In 1 confirmation set, isolated leukocytes were used, and in the second, whole blood was used. Although 11 of 14 genes were found to have similar increases in expression, this may have been because of the specific limited genes chosen and the small size of the data sets.

It has become clear over the past few years that information obtained from gene expression data is vital to discovering new mechanisms for disease and has clearly aided diagnosis and treatment in the field of oncology. Although less is known in relation to cardiovascular disease, its inherent complexity makes the vast amount of data available from transcriptomics methods both exciting and daunting with regard to the goals of diagnosis and treatment. Blood samples are readily available from patients and hold the promise of utility in the understanding, treatment, and diagnosis of vascular and thrombotic diseases; however, much remains to be clarified merely to effectively utilize these methods. It is crucial that our knowledge base not be diluted by underpowered studies, lack of diligence in addressing technical and scientific details, and failure to use appropriate statistical information to provide invaluable biological information that will address the biological complexity of cardiovascular disease at a new and intricate level.

Sources of Funding
Dr Freedman’s research is supported by the National Institutes of Health/National Heart, Lung, and Blood Institute.

Disclosures
None.

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**Key Words:** Editorials • blood cells • cardiovascular diseases • genes • genetics
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doi: 10.1161/CIRCGENETICS.108.814186
Circulation: Cardiovascular Genetics is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1942-325X. Online ISSN: 1942-3268

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