Gene Expression Profiling in Hypertension Research
A Critical Perspective

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Abstract—Recent advances in molecular biology and technology have made it possible to monitor the expression levels of virtually all genes simultaneously. As the tools for gene expression profiling have become more widely available, the number of investigators applying this technology in hypertension research, as in other fields of biomedical research, has grown rapidly. At the same time, numerous articles have been published that discuss the technical aspects of gene profiling and its promise for advancing research on the pathogenesis and treatment of multiple clinical disorders. However, much of the research carried out with gene expression profiling has been of a correlational or descriptive nature, and the true value of this technology is unclear. Despite the initial wave of enthusiasm for gene expression profiling, its actual utility for studying multifactorial disorders like hypertension remains to be established. In this review, we offer a critical perspective on the use of gene expression profiling in hypertension research and discuss some emerging strategies for taking this technology beyond the limits of correlational and descriptive studies. (Hypertension. 2003;41:3-8.)

Key Words: gene expression ■ hypertension, essential ■ gene expression ■ genes ■ DNA

When it comes to trying out cutting edge technologies such as cDNA microarrays and other methods for gene expression profiling, investigators studying hypertension are no different from scientists in other fields of research; we end up getting caught in the hype cycle. The term hype cycle was coined in the mid 1990s to describe the sequence of events that often occurs when an exciting new technology is introduced (Figure 1).1,2 This sequence of events is characterized by an initial wave of unbridled enthusiasm and a peak of inflated expectations followed by a trough of disillusionment because the technology does not live up to its initial promises. The period of disillusionment is then followed by more focused experimentation and critical work efforts that lead to a truer understanding of the technology’s applicability and utility. Eventually, a plateau of productivity occurs as the genuine benefits of the technology become established, with the final height of the plateau being determined by whether the technology is broadly useful or only benefits niche applications. In this review, we discuss the use of gene expression profiling in hypertension research with an eye on where this technology fits in the hype cycle and where it may be headed in the future.

A variety of methods is now widely available for quantifying and analyzing gene expression on a genome-wide basis, including cDNA and oligonucleotide microarrays and serial analysis of gene expression (SAGE). The technical advantages and disadvantages of the various methods of gene profiling and data analysis have been discussed in detail elsewhere and will not be reviewed here.3–10 Issues related to statistical methods, gene profiling of tissues containing mixed cell populations, transcriptional or posttranscriptional changes undetected by gene profiling, and the need to replicate results will also not be discussed. Clearly a number of important issues of this nature remain as the technology continues to mature, particularly in the rapidly growing area of bioinformatics and data management.4,7,8 This review will focus on strategic questions about the use of gene expression profiling in hypertension research rather than engineering and statistical issues related to the technology itself.

Fishing Expeditions Versus Hypothesis-Driven Research
Increasing numbers of investigators are making global measurements of gene expression in studies pertinent to hypertension and related cardiovascular disorders.11–16 One of the most common experimental designs compares gene expression levels in experimental models with differing levels of blood pressure or after exposure to various stimuli that affect blood pressure. Gene expression profiling of animals with different degrees of target organ damage is also becoming increasingly common as are studies of cardiovascular cell lines and tissues subjected to different experimental conditions.3,17 Invariably, these studies generate long lists of genes...
that are differentially expressed between the experimental groups, leading investigators to conclude that the phenotype of interest is associated with alterations in expression levels of these sets of genes. Included in these sets are some genes whose expression levels could have been predicted to be affected by the phenotype under study and others for which changes in expression levels could not have been anticipated. This applies particularly to expression profiling with expressed sequence tags, representative of genes whose functions are entirely unknown. After reading these kinds of studies, one may feel compelled to ask, “So what?” Indeed, studies based on large scale gene expression profiling have been criticized as fishing expeditions that often test an uninteresting hypothesis or no hypothesis at all.

Although it is true that many gene profiling experiments are not hypothesis driven, the rejoinder to this criticism is that such studies can be highly valuable in generating new questions and hypotheses. By categorizing groups of genes according to the expression patterns they show across a wide range of experimental conditions, this technology can provide clues about the function of novel genes based on the expression categories to which they belong and the functions of known genes that fall within those same categories. Many examples of this exist in the literature in other, diverse areas of biology, for example, in the classification of cancer and in defining metabolic networks in simple organisms such as yeast. In cardiovascular research, microarray technology has also identified genes whose expression levels show unexpected relationships to blood pressure, target organ damage, or other cardiovascular phenotypes of interest. The identification of such genes in turn generates a whole new set of hypotheses pertaining to their potential roles in disease pathogenesis, prevention, or management. For example, a recent expression profiling study by Okuda et al detected a marked increase in cytosolic epoxide hydrolase (Epdx2) mRNA in a hypertensive rat model, which, along with the finding of allelic variants between spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats and demonstration of altered blood pressure in Epdx2 mice, led to new hypotheses about the role of this gene in hypertension pathophysiology and the potential of Epdx2 as a drug target.

From studies such as this, the hope has arisen that even mundane gene profiling experiments that simply correlate changes in gene expression with hypertension-related phenotypes will eventually succeed in identifying some new pathways of functional significance or perhaps even lead to the identification of the causal genes involved in the primary pathogenesis of hypertension. However, the likelihood is that many gene profiling experiments of a correlational or descriptive nature will end up being viewed like most studies of multifactorial phenotypes, ie, questions of cause and effect will remain unclear, and more powerful and focused strategies will be needed to untangle the complex web of relationships.

Beyond Correlation Studies

The need to move beyond conventional correlation studies and seek out primary mechanisms of functional significance had challenged hypertension scientists long before the advent of gene profiling technology. The literature is replete with reports claiming associations between hypertension and almost every physiological and pathophysiological variable that one can imagine. With the sequencing of the entire human genome, we now have thousands upon thousands of additional variables, ie, DNA polymorphisms and gene expression profiles, that are being subjected to association/correlation studies in hypertension.

In contrast to simple DNA polymorphisms, gene expression levels will most often represent complex, quantitative phenotypes determined by multiple environmental and genetic factors. The main advantage of gene expression profiles over measurements of other complex phenotypes is that one can monitor thousands of different mRNA phenotypes simultaneously. However, given the problems interpreting association studies of hypertension and DNA polymorphisms, one begins to appreciate the challenge of interpreting studies of gene
expression levels that can be influenced by blood pressure and a host of other environmental and genetic factors.

Notwithstanding the well-established role of the kidney in the long-term regulation of blood pressure, it is also uncertain which tissues and cell types are most important to study with respect to the primary pathogenesis of hypertension and, therefore, which will most likely yield useful gene expression profiling results. And regardless of which tissues are chosen for study, the key question is whether any of the measured changes in mRNA levels are truly relevant to hypertension, and if so, whether they are involved in disease pathogenesis or simply represent secondary responses to the increased blood pressure. Until recently, many investigators performing conventional gene profiling experiments have not been forced to address the tough questions that are ordinarily put to those conducting correlation studies in hypertension or comparisons between subjects with differing levels of blood pressure. However, as the number of gene profiling studies increases, such questions will be asked on a more frequent basis, with less validity being given to descriptive experiments that simply categorize changes in gene expression that may be associated with altered blood pressure. Hopefully, as gene profiling enters into a later phase of the hype cycle, we will gain a better understanding of the true value of this technology for studying complex disorders like hypertension.

Time Course Studies for Testing the Functional Relevance of Gene Expression Levels in Hypertension: How Useful?

One strategy frequently used in dealing with issues of cause and effect in studies of hypertension is to identify phenotypic changes that precede the onset of increased blood pressure or target organ damage. However, investigators using this approach in gene profiling studies will be faced with the same challenges encountered by those who have struggled to identify and determine the relevance of changes in other variables that may occur before the onset of hypertension or target organ damage. For example, the literature contains many studies purporting to show changes in certain variables before the onset of increased blood pressure in SHR. A recent gene expression profiling study of vascular smooth muscle cells falls into this category. Unfortunately, the value of this experimental approach is uncertain for a number of reasons, including the fact that blood pressure may be increased in SHR at birth, if not earlier. Even in Dahl salt-sensitive rats, blood pressure also appears to be increased at a very young age despite administration of a low-salt diet. Even in animal models in which hypertension is experimentally induced, the time of onset of increased blood pressure or target organ damage may be difficult to establish in relation to the time of onset of changes in gene expression. The failure to measure blood pressure continuously in conscious animals and the failure to use sensitive techniques for detecting target organ damage will undoubtedly lead to some false-positive results. Moreover, even if one succeeds in clearly identifying a change in gene expression that precedes the onset of an increase in blood pressure or the onset of cardiovascular injury, the identification of such a change does not prove its role in disease pathogenesis.

Combining Genetic Strategies With Genome-Wide Expression Profiling

Nearly 20 years ago, Rapp pointed out the limitations of studies that simply compare hypertensive and normotensive strains with respect to different quantitative traits and emphasized the value of genetic paradigms for investigating the pathophysiology of complex phenotypes like essential hypertension. Rapp’s approach represented an attempt to move beyond the usual correlation or comparison studies involving hypertensive animals and normotensive controls and, together with advances in DNA genotyping, has led to the identification of multiple chromosome regions (quantitative trait loci, QTL) linked to the regulation of blood pressure. Unfortunately, despite the obvious advantages of linkage strategies and the advances in genetic typing and mapping technologies, overall progress has been slow, and only a few of these blood pressure QTL appear to have been identified at the molecular level. However, one strategy that has been used successfully to accelerate the search for genes underlying various phenotypes related to cardiovascular disease is to combine genetic strategies with genome-wide expression profiling. By interpreting genome-wide expression profiles in a genetic context, it may be possible to extract more information from the technology than is usually gained from conventional studies of a more descriptive or correlational nature. Accordingly, it is hoped that the combination of genetic strategies with genome-wide expression profiling will be helpful in unraveling the pathogenesis of complex disorders like hypertension.

Applying Gene Expression Profiling in Genetically Selected and Modified Strains

One approach to the combined use of gene expression profiling and genetic strategies is to apply gene profiling in genetically selected animals, such as congenic strains, or in strains that have been manipulated using transgenic techniques. This approach has a number of advantages over gene-profiling studies of hypertensive and normotensive strains that harbor a large number of genetic differences likely to be of little or no pathologic relevance to hypertension. Interpretation of gene expression profiling studies of 2 strains that are genetically diverse can be complex because such comparisons may yield differences in the expression levels of very large numbers of genes. For example, in studies comparing conventional SHR and normotensive rats (eg, Wistar-Kyoto or Brown Norway [BN] strains), approximately 1% of genes tested in the heart, brain, kidney, or adipose tissue were considered to show significant differences in expression between the strains. Thus, we can expect hundreds of genes to be differentially expressed in comparisons between conventional strains of hypertensive and normotensive rats. In contrast, comparisons between strains that have a limited number of well-defined genetic differences can reduce the complexity of the gene profiling analysis and help guide the focus on high-priority candidates for follow-up studies.

The application of gene expression profiling in genetically selected models of cardiovascular disease is illustrated by the recent use of cDNA microarray analysis in congenic strains to search for gene variants contributing to susceptibility to metabolic features of Syndrome X. In these studies, gene profiling was performed in a congenic strain of SHR that is
genetically identical to the SHR/National Institutes of Health (NIH) progenitor strain except for a single segment of chromosome 4. The SHR congenic and progenitor strains are known to exhibit differences in susceptibility to dietary-induced insulin resistance and dyslipidemia, as well as differences in other phenotypes related to the hypertension metabolic syndrome.37 The studies revealed a gene that was differentially expressed in adipose tissue of the 2 strains and that mapped directly within the congenic region of chromosome 4, known to harbor a QTL contributing to features of Syndrome X in this model.11 This gene turned out to encode the CD36 fatty acid transporter, and transgenic experiments confirmed that defective Cd36 contributes to dietary-induced insulin resistance and increased serum levels of fatty acids in the SHR/NIH strain.38 Subsequently, other investigators found that mutations in Cd36 are also associated with insulin resistance and dyslipidemia in humans.59 Compared with the use of conventional hypertensive and normotensive strains, the use of congenic strains in this study reduced the number of differentially expressed targets by 80%.11 Moreover, by concentrating on genes that were not only differentially expressed but that also mapped within the congenic chromosome segment, it was possible to narrow the focus of the gene-profiling results even further. Indeed the locations of a large subset of differentially expressed genes between SHR and BN have now been determined.40 allowing investigators to examine this data set for genes that map to chromosomal regions of interest for any given phenotype.

Although gene expression profiling of genetically selected and modified strains can offer advantages over expression profiling of conventional hypertensive and normotensive strains, this approach is not without limitations. For example, in some cases and regardless of the types of strains tested, a gene variant may contribute to hypertension without clearly affecting expression of the gene itself. Such a variant will not be directly revealed by gene expression profiling; however, it is possible that it might alter the expression patterns of other genes, which in turn could give clues to the identity of the primary defect. Expression profiling of gene-targeted strains may be particularly helpful in testing for downstream gene pathways and mechanisms triggered by a specific gene modification of interest. Nevertheless, just as with gene-profiling studies in conventional strains, changes in gene expression in genetically modified strains do not necessarily reflect primary genetic mechanisms contributing to disease pathogenesis. Changes in gene expression could result from (1) strain differences in other phenotypes, (2) irrelevant genetic variants physically linked to the primary gene of interest, or (3) other effects mediated by the primary gene defect that are unrelated to disease pathogenesis. It is interesting to note that the Sce gene was identified as a positional candidate gene for a QTL in SHR on the basis of expression profiling by cDNA subtraction analysis.41 However, the creation of congenic strains has excluded Sce as the causal gene for this QTL.42–44 Thus, further experiments will often be required to investigate whether any observed changes in gene expression are likely to reflect primary pathogenetic mechanisms, as opposed to secondary or unrelated phenomena. Examples of such experiments include the use of gene expression profiling across a panel of genetically modified strains or the use of gene expression profiling in tissues maintained in the same host in vivo or in the same environment in vitro.45–46

Interpreting Gene Expression Profiles in Light of Genetic Linkage Studies of Cardiovascular Phenotypes

A simple example of how genetic linkage studies can be used to help guide interpretation of gene expression profile data is illustrated by one of the first gene-profiling experiments to be conducted in an experimental model of hypertension. In this study,15 genome-wide expression profiling of heart, brain, and kidney tissue by a proprietary gel-based method was used to search for genes that are differentially expressed between SHR and the stroke-prone SHR (SHRSP). The gene-profiling results were then analyzed in combination with the results from published linkage studies to identify positional candidate genes for cardiovascular phenotypes such as hypertension or stroke susceptibility/stroke resistance. Using this approach, Shimkets15 found that the Nppa gene encoding the precursor for atrial natriuretic factor was differentially expressed in the brains of SHR versus SHRSR and noted that Nppa mapped to a region of rat chromosome 5 previously linked to a stroke-related phenotype in the SHR/SHRSP model. DNA sequencing has revealed a number of molecular variants in the Nppa gene of the SHRSR and SHR strains, and Rubattu and colleagues47,48 have proposed that, by influencing Nppa transcription and atrial natriuretic peptide (ANP) levels in the brain, one of these variants may be influencing the pathogenesis of stroke.

Although more studies are needed to definitively determine whether variants in Nppa truly influence susceptibility to stroke, Shimkets’ study illustrates a simple way in which the use of genetic linkage data can be used to guide analysis of gene expression profiles. Of course, based on the genetic mapping data and the known biology of ANP, it was possible for investigators to identify Nppa as a potential gene related to stroke without the use of gene expression profiling. However, the combination of gene profiling and genetic data can also be used to direct attention to target candidate genes or pathways that may not be obvious and thus facilitate the identification of novel mechanisms involved in disease pathogenesis. Another example of the combined use of gene profiling and genetic mapping data to identify a disease gene pathway is the study of Lawn et al13 who used this approach to identify a defect in the ABCA1 gene as the cause of Tangier disease, a monogenic disorder characterized by high-density lipoprotein deficiency and increased risk for atherosclerosis. The hope is that similar strategies will be useful for narrowing the focus on gene variants involved in the pathogenesis of more common forms of cardiovascular disease, such as hypertension. It should be recognized, however, that the utility of this approach in studies of multigenic forms of experimental hypertension could be limited because of the increasing number of chromosome regions that are reported to be linked to the regulation of blood pressure. Moreover, the confidence intervals that delineate these regions are typically quite large, and without the availability of congenic lines and sublines, it is difficult to narrow the map locations of blood pressure regulatory genes
with a high degree of precision. If every chromosome is reported to harbor extensive regions linked to the regulation of blood pressure, mapping results from linkage studies may not in themselves be very useful in focusing the analysis of gene-profiling experiments. The progressive restriction of chromosomal regions, although slow, will eventually allow more precise testing of positional candidates, however identified, purely on the basis of chromosomal location. This will be of particular importance for positional candidates identified by expression profiling.

**Genetical Genomics: Linkage Analysis of Gene Expression Profiles**

Because the expression level of any gene can be treated as a phenotype, it is possible to use linkage paradigms to map chromosome regions involved in the regulation of gene expression. Linkage analysis of gene expression levels in segregating populations represents an approach to combining the power of gene profiling and genetic analysis on a global scale and has been termed *genetical genomics.* In fact, by monitoring gene expression profiles and blood pressures in an F2 population or in recombinant inbred strains, one can perform a genome-wide search for target chromosome regions linked to the regulation of both blood pressure and gene expression levels. This strategy involves searching for correlations between gene expression patterns and blood pressure and, just like other correlation studies, may reveal variations in gene expression that are simply secondary to variations in blood pressure. However, with this strategy one can quickly identify any gene that physically maps within a chromosome region that is linked to the regulation of blood pressure and the expression level of that same gene (Figure 2). This approach may afford powerful opportunities for identifying variants with pleiotropic effects on gene expression levels and blood pressure and decrease the chances of focusing on irrelevant strain differences in gene expression occurring as a result of genetic drift. As discussed by Jansen and Nap, genetic analyses of gene expression profiles may be particularly helpful in unraveling the genes and gene products that are involved in complex metabolic and regulatory pathways. Thus, we anticipate that as gene expression profiling becomes more widely used in cardiovascular research, it will be increasingly applied in a genetic context for many of the same reasons that motivated Rapp to develop genetic paradigms for the investigation of hypertension.

**Gene Expression Profiling and the Hypertension Clinic**

It is far too early to tell whether gene expression profiling will be of any practical value as a diagnostic or management tool in the hypertension clinic. However, it is only a matter of time before someone attempts to determine whether gene expression patterns in peripheral blood cells or some other readily accessible tissue can be used to guide the classification or management of patients with essential hypertension. Such data will require evaluation alongside established treatment protocols and comparison with other new strategies for hypertension management, including the use of pharmacogenetics, for example, through the use of genotyping at polymorphic sites in relevant genes. Given that multiple environmental and genetic factors determine the blood pressure response to antihypertensive therapy and individual susceptibility to target organ damage, the potential clinical value of gene expression profiling in managing hypertension is unlikely in the short term and remains to be determined in the longer term.

**Summary: Gene Expression Profiling and the Hype Cycle**

Although investigators who are just starting to use gene expression profiling may have considerable enthusiasm and high expectations for the technology, many of those with experience have already been through a period of disillusionment in the gene profiling hype cycle (Figure 1). Some seasoned investigators are attempting to develop more focused and productive ways to use this experimental tool; however, additional time will be required before the genuine benefits and value of gene profiling become clear and it reaches a final plateau in the technology hype cycle. In this regard, we anticipate that the combined application of gene profiling and genetic strategies will emerge as one of the more valuable approaches to facilitating the intelligent and productive use of this technology in the near future.

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