Express yourself—But consider the consequences

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In less than its allotted 15 years, the US Human Genome Project has produced all of its promised products and then some, including a complete draft (minus a few pesky hard-to-clone regions) of the sequence of the 3.2 billion nucleotides of DNA found in each haploid set of human chromosomes. The publication of this draft sequence coincides with the 50th anniversary of the famous and famously brief letter to *Nature* by James Watson and Francis Crick¹ in which they proposed the double-helix model of DNA structure. The human genome sequence, not to mention the sequences of the mouse and a host of other eukaryotes and prokaryotes, represents an enormous amount of information, only a fraction of which has been analyzed. Indeed, the work of validating, cataloguing, comparing, and interpreting genetic information has spawned a growth industry in the field now termed bioinformatics. Another derivative of the various genome projects around the world has been rapid advances in bioinstrumentation. The most obvious example is the evolution of automated machines that churn out nucleotide sequence data; the speed of sequencing increased almost in proportion to the decrease in cost per nucleotide.

Other technologies evolved quickly as well. The first demonstration of hybridizing samples of RNA or DNA in solution to short nucleotide sequences (oligonucleotides) fixed to a glass slide occurred a decade ago.² The platforms became known as arrays or chips, and a variety of techniques was developed to construct them. One popular technique combines photolithography with solid-phase chemistry. Tens or hundreds of thousands of different molecular probes can be affixed in defined order to a support the size of a microscope slide cover slip. The probes can be nucleic acids, proteins, or other molecules. Many are now commercially available, and one can order, for example, a chip to which are affixed oligonucleotides representing all of the messenger RNAs known to be expressed in the normal human liver. The processes of enabling hybridization with a test sample, detecting hybridization (usually by means of a fluorescent tag), recording the signals, and analyzing the results have been automated. Thus the technique is within reach of any laboratory scientist with the funding to purchase either the equipment or the services of an institutional core laboratory. A single experiment produces an enormous quantity of data, and managing and interpreting the results have provided further job security for those who practice bioinformatics. Array technology is being applied to a multitude of purposes, including DNA sequencing, detection of mutations, drug discovery, and comparison of one person's, or one species', genome with another. During the mid-1990s, a handful of articles was published on array technology. For January 2003 alone, a MEDLINE search found over 250 articles using oligonucleotide arrays.

The article by Absi and colleagues³ in this issue of the *Journal* illustrates one application of oligonucleotide arrays, expression analysis. At the most fundamental level, cells of different tissues differ from one another genetically in terms of which genes are active, or expressing, at a given time. Gene expression produces RNA; in some instances, RNA is the final product, but usually the RNA is a message that gets translated into a protein. The amount of final product of gene expression present in a tissue or an organ reflects both the rate of transcription of RNA from the gene and the stability of this message. In the first instance gene expression is controlled by the fundamental mechanisms of development and differentiation. However, tissues and organs, especially when they are of interest to physicians, are rarely pristine in terms

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of which genes are active and, for those that are active, how vigorously they are being transcribed. Pathologic processes, whether intrinsic (eg, malignancy) or extrinsic (eg, infection), activate some genes and suppress transcription of others. In turn, attempts by the body to remedy the pathology (eg, immune responses and fibrosis) affect the expression of yet other genes. For any given disease in any given tissue, an effort to analyze gene expression, either qualitatively or quantitatively, must account for all of these effects.

This creates a conundrum for those surgeons who are interested in the causes and pathogenesises of the diseases they treat and have access to impressive quantities (from a molecular biologist's perspective) of diseased tissue. If one examines the genes that are turned on and off in a surgical specimen, the result will often reflect the end stage of the pathology, in which much of what is observed is a reaction to, rather than the cause of, the disease. Controls are crucial when comparisons are made among patients, with age, sex, and stage of disease being obvious parameters. But difficulties persist in studying a single patient: simply analyzing a specimen of adjoining normal tissue will not solve the problem of distinguishing epiphenomena from real pathogenesis. Most progress with expression arrays is being made in situations in which the histologic progression is certain and samples can be obtained from all intervening stages. For example, within a single surgical field, a patient with familial adenomatous polyposis might exhibit normal colonic mucosa, mucosal dysplasia, adenoma, adenocarcinoma, and metastasis.

Unfortunately, for those of us interested in aortic diseases, neither this neat pathologic sequence nor the availability of contiguous informative specimens is feasible. Moreover, the aorta, throughout its length, is not a homogeneous organ in terms of embryology, physical elastic properties, and biochemical composition. The histopathologic characteristics of the most common form of aneurysm in the ascending aorta (ie, medial degeneration, previously and incorrectly termed "cystic medial necrosis") differ markedly from those of the typical abdominal aortic aneurysm (inflammatory and atherosclerotic). Recent studies of gene expression in resected specimens of abdominal aortic aneurysm have revealed considerable differences when compared with nondilated specimens.^{4,5} Increased expression of a matrix metalloproteinase came as little surprise because immunohistopathologic studies had indicated increased levels of this protein in the aneurysm wall. However, what increased expression of, for example, CD86 antigen and decreased expression of neuronal pentraxin II mean about the pathogenesis of abdominal aortic aneurysm remain anyone's guess. A recent study comparing expression in acute ascending aortic dissection with that in aortas from organ donors leaves the same sense of frustration.⁶

The article by Absi and colleagues³ tackles a much larger purview: comparing aneurysms in quite distinct regions of the aorta. Given the marked epidemiologic and histopathologic distinctions between aneurysms of the ascending and abdominal aortas, the finding of markedly different expression profiles comes as no surprise. However, as the authors caution to some extent, the differences might reflect more than distinct causation. First, the ages of the subjects were highly varied, with younger patients disproportionately represented in the ascending aortic specimens. To what extent do the expression patterns in the abdominal aortic tissue represent aging phenomena as opposed to pathology specific to aneurysm formation?

Second, patients with abdominal aortic aneurysms frequently have intercurrent illnesses, both chronic and acute. Can the expression profile be altered by these factors compared with the profile in pure aneurysmal disease (if this exists outside of rare hereditary disorders)? The best control specimens need to be matched not only for specific anatomic location (ie, infrarenal vs suprarenal), age, and sex but also for blood pressure, smoking status, degree of atherosclerosis, medication use, and so forth.

Third, the numerous contributors to experimental variability need to be a constant concern. Most arrays contain multiple spots for each probe, and the results from several hybridizations are averaged. However, there has been little attention to variability within a single specimen from the same patient. The investigation of Absi and colleagues³ provides little information with regard to intraindividual variation and demonstrates a fair amount of interindividual variation among patients with the same histopathologic appearance.

Finally and most importantly, the data are descriptive. To be fair, hypothesis-driven experiments on aortic diseases currently can only be done in animal models or in vitro by using cultured human cells or pieces of tissue,⁷ all of which could be of limited relevance to human disease.

We now possess a vast array of data (witness Tables 1 and 2 of the article by Absi and colleagues³) about differential gene expression in different forms of aortic aneurysm, but what the data tell us about cause and pathogenesis remains to be elucidated.

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