October 2002 Issue | Dr. Brad Rachman, GSDL Great Smokies Diagnostic Laboratory

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Welcome to Functional Medicine Update for October 2002. In this issue, we will focus on the use of the clinical laboratory in functional medicine, a topic we have not fully covered in any single issue in the last 20 years. It is an important and timely topic in regard to the application of functional medicine in the clinic.

What objectives are we are trying to achieve in the clinical laboratory? Obviously, we want to improve our understanding of the underlying mechanisms, etiology, pathogenesis, and ultimate sequence of events that are associated with a specific illness. In the past, the medical model regarding the use of the clinical laboratory was fairly simplistic. We looked for the markers of end-organ pathology in a specific biological specimen or fluid. The level of a specific analyte would indicate the tissue had undergone some kind of necrotic or apoptotic cell death.

Pathological Markers of Cell Death
For example, with liver injury, as in hepatitis or cirrhosis, we look for elevations of specific enzymes that are markers of liver cell function, hepatocyte function. Transaminases represent one such marker. The liver is heavily responsible for manipulating amino acids, through transamination and deamination. Therefore, there is a high level of activity of these transaminases in the liver cell. When the liver cell dies and releases its contents into the plasma, we can use this surrogate marker, which is the high level of transaminase enzymes in the plasma, as an indication of the degree of liver death.

When we talk about liver cell death, or heart cell death, or muscle cell death, the pathological evaluation is to look for specific markers indicating that cell has experienced injury or death. This is a tidy way to use the laboratory to diagnose a disease, as if that disease were caused by a specific injurious factor that led to a whole-organism or whole-organ effect.

Functional Changes Preceding Cell Death
Well before cell death occurs, however, in the sequence of events that leads to tissue pathology, are many behind-the-scenes variables associated with altered cellular function that ultimately lead to cell death. A search for these precedent factors, often the functional changes in cells as a consequence of alterations in gene expression are what drive the functional medicine paradigm. Cell function is related to these precedents that alter the expression patterns of gene activation, and are ultimately revealed as a perturbation of cellular physiology that leads to premature cell death.

An example may help clarify this point. At the beginning of the 20th century, a major medical advance occurred that guided much of the development of medicine. That advance concerned the understanding of infectious organisms as the cause of infectious diseases. The model of disease at that point was reasonably easy to understand. A single agent, e.g., an organism like pneumococcus, produces a single disease, e.g., pneumonia. It was later discovered that a single molecule could treat that disease. That medical model was very simple. A single agent produces a single disease for which a single molecule, i.e. a pharmaceutical, is used to treat it.

If medicine had stayed that simple over the last 100 years, it wouldn't take advanced schooling to get a medical degree. In fact, you could memorize a comprehensive list of single agents causing single diseases for which single molecules would provide treatment, and you could be a successful physician.

Complex Multi-Gene Diseases

The difficulty, however, is that the diseases that have become predominant in an aging Western population are not as simple as single-agent/single disease/single molecule treatment. Instead, they are complex diseases of multiple genes in which genetic susceptibility factors interact with a complex environment to give rise to a phenotype we call disease. Examples include coronary heart disease (CHD), cancer, maturity-onset diabetes, osteoarthritis, inflammatory bowel disease (IBD), certain behavioral abnormalities, psychiatric disorders, and neurodegenerative disorders. All reflect the new model of medicine, the interaction of numerous genes with environmental factors giving rise to an altered phenotype.

This new model is much more complex and difficult to understand. Instead of looking for single disease indicators, such as elevated liver enzymes that identify such liver injury as hepatitis or cirrhosis, we are looking for the mechanisms that give rise to altered cellular activity and, ultimately, the dysfunction we call disease. That is the functional medicine model.

Standard Blood Screens

The clinical laboratory was not well set up, in its early stages, to answer these questions. Therefore, while many people used the standard blood screens, the SMA or comprehensive screening systems, in an attempt to evaluate functionality the screens, however, were set up to evaluate pathology not function. If you took blood glucose levels, BUN to creatinine levels, serum AG ratios, albumin-to-globulin ratios, SGOT/SGPT CPK, or other analytes related to calcium or phosphorus, and sodium or potassium, they would help you understand the pathological state of the cell, but not its function.

Evaluating Risk Factors with Blood Screening

Some of those analytes are related more to functionality than others. One that was a later addition to the panel was cholesterol. No single disease exists for which we can say cholesterol is an analyte. It is a prognostic factor relating to risk for a variety of vascular disorders. The addition of cholesterol, then later the subtypes of apolipoproteins of cholesterol like HDL, gave rise to a new way of utilizing the blood pathology screen. That was to evaluate relative risk rather than just existing pathology. There is no single disease that we can say is a cholesterol disease. Rather, cholesterol/HDL ratios are used as prognostic evaluative risk factors for later-stage vascular disorders.

The focus of the clinical laboratory has now changed from pathology to prognostic or risk factor analysis.

Functional Screening in the Laboratory
Moving ahead another step, we consider using the clinical laboratory in evaluating functionality at the cell physiological level. Even further, we can use it to evaluate the function resulting from the pluripotentiality of the genome as information flows from the genes to proteins . This is the promise afforded by proteomics.

The Many Focuses of the Clinical Lab
Therefore, depending on what questions you ask, the clinical laboratory can be used in many ways. Traditionally, because we have focused almost exclusively on diagnosis as a prima facie requirement for good medicine, (the sine qua non is to come up with a good diagnosis), the clinical laboratory has focused on helping us make a clean diagnosis from a pathological perspective. We look at such things as antinuclear antibodies for the presence of various autoimmune disorders, for example, or prothrombin time to look at coagulation or sedimentation effects relating to blood coagulation parameters. Or, we examine various markers and electrolytes, potassium and sodium levels, to evaluate adrenal function and look for endocrinopathies or specific pathologies of the endocrine system.

In an aging population whose declining function is associated with chronic disease, however, the major challenge to medicine is managing these chronic diseases rather than waiting to make a clean diagnosis of a disease that requires hospitalization, surgery, and other costly interventions. That is the concept of using the clinical laboratory in assessing function from a functional medicine perspective.

This new model is based on genetic multiplicity based on nucleotide polymorphisms. Interactions with a complex environment then give rise to different kinds of disorders that later become disease entities. Understanding this sequence of events and the mixture of environmental factors with genetic uniqueness has resulted in the new field of personalized medicine

This theme is the subject of a recent issue of *Science* magazine, the cover title of which is "The Puzzle of Complex Diseases." The title of the introductory editorial to this series of articles is “It's Not Just the Genes." The authors of this editorial point out that complex diseases involve genetic susceptibility or genetic uniqueness combined with environmental exposures and environmental factors including toxins, trauma, environmental effects, radiation, and psychosocial variables including distress, allergies, and chronic infections.

Exposure of the genes to these various factors gives rise to different expression patterns that translate into different physiological phenotypes at the cellular level. It is the laboratory's emerging responsibility to understand how these complex interactions ultimately translate into later-stage diseases.

Disease as a Phenotype
One article in this series in *Science* is titled "Maneuvering in the Complex Path from Genotype to Phenotype." The author of this article talks about human disease as a phenotype.[2] It is the end stage of a series of events controlled not only by genes, but by a self-organizing network that displays system-wide dynamics. These networks, which we see as metabolic pathways, respond to signaling events that occur at the cellular level. These events derive from an interaction of the environment with the genes. The signals extend to cells, tissues, and organs at a distance, and there are different responses of the body to that state effect.

Study of the dynamics of these networks of approaches has led to metabolic control analysis, which people are now using to gain new insights into the pathogenesis and treatment of complex disorders we call chronic illness, which may not be amenable to a very clean diagnosis.

A number of times in your experience, you may have had a patient come in with an array of symptoms ranging from headaches to digestive problems to chronic fatigue, to muscle body pain such as myalgias, to joint space pain, to cognitive dysfunction, to inability to concentrate, and sleep disturbances. It would be nearly impossible to find a single clean diagnosis for this complex array of symptoms. Their phenotypes reflect a reaction of the genes to the environment in such a way as to create these complex signals related to metabolic dysfunction, or metabolic loss of control, or a different state function of their metabolism, which is a state function of chronic illness.

The Clinical Lab and the "Omics" Revolution

In metabolic control analysis, we look at the metabolome, this complex series of events we see in metabolism that derive from the proteome, the enzymes and proteins that control metabolism. We then try to determine how that relates to the genome in this new field of genomics, proteomics, and metabolomics, the so-called "omics" revolution with which we are now involved.

This field represents a new use of the clinical laboratory to evaluate functional genomics, functional proteomics, and functional metabolomics. We are trying to understand the trajectory and tendency toward specific diseases that, along the way, are producing complex symptoms of dysfunction that lead to the necessity for medical services that may have been unnecessary if we understood the origin of these conditions

With one example we can take this discussion from the philosophical level to a reality base. That example, which we have discussed in previous editions of *FMU,* is the folate, vitamin B12, and homocysteine story that is emerging around the genetic polymorphisms of the folate cycle enzymes. We have focused on one of these enzymes that shows considerable genetic variation with high penetrance. It is the enzyme called methylenetetrahydrofolate reductase (MTHFR).

MTHFR catalyzes one of biochemical steps in the folate cycle and the homocysteine cycle. It is involved principally in setting in motion the transmethylation process that results in the transfer of methyl groups to produce complex phospholipids for the nervous system, for cellular membranes, for steroid hormone manipulation, neurotransmitter detoxification, and metabolism. It is an important general one-carbon metabolic pathway, as well as the clearance of homocysteine.

MTHFR Polymorphisms

As we have stated in previous editions of *FMU*, between 20 and 30 percent of the population in America has been found to have polymorphisms, or so-called single nucleotide polymorphisms (SNPs) of the methylenetetrahydrofolate reductase gene. A common SNP is a polymorphism that substitutes a cytosine for a thymidine at residue 677 in the messenger RNA (mRNA) derived from that gene. It in turn encodes a particular variant of the enzyme with a demonstrable effect on phenotype. Nucleotide 677 in the mRNA derived from the MTHFR gene can exist in one of two forms. It can exist in the wild-type form (T), which is the common allele found in most of the population. Less commonly, it can exist as the variant (C) which in turn can be present in either the heterozygous (C/T) or homozygous (C/C) meaning it was transmitted as a single allele from only one parent, or inherited as the double allele, one copy coming

from both parents. The homozygous ($677^{C/C}$) individual exhibits the characteristic phenotype much more commonly than heterozygous ($677^{C/T}$) individuals.

There are variations on a theme. There is the wild type; there is the fully-fledged homozygous type with both alleles, both mother and father's contributions showing 677T; and there is the halfway point, 50 percent showing 677 and the other half not.

S-Adenosylmethionine (SAM)

What this means is that you may get variations in phenotype with regard to the way an individual can metabolize folic acid and transfer methyl groups into the homocysteine pathway to makeS-adenosylmethionine (SAM), the principal methyl donor. Defects in this particular pathway can have multiple effects on different diseases. The list, as you heard in previous editions of *FMU*, is quite long. It can include not just heart disease and stroke, but also risk for breast cancer, endometrial cancer, possibly prostatic cancer, depression, various disorders related to neurodegenerative diseases, perhaps even Alzheimer's, and conditions related to various epithelial malignancies, including colon cancer, cervical cancer, and possibly lung cancer.

The methylation story plays across many different disorders that have a single diagnosis. This mechanism may cut across many diseases. It may relate, in part, back not just to MTHFR polymorphisms, but to this polymorphism in combination with others. Remember, multiple genes, along with multiple environmental factors, give rise to complex disorders. This is the emerging view of disease, at least the common kinds of chronic degenerative diseases we find with age-related dysfunctions.

The recent editorial in the *American Journal of Nutrition,* titled "Methylenetetrahydrofolate Reductase: a Link between Folate and Riboflavin?" discusses this enzyme process associated with methyl donation through the MTHFR enzyme and explains that it also depends on flavin adenine dinucleotide (FAD), derived from riboflavin by vitamin B2.[3] Therefore, there is a strong correlation among MTHFR polymorphisms, not only folic acid status, but also riboflavin status. Certain folic acid derivatives, such as 5-methyltetrahydrofolate, which works downstream from this enzyme polymorphism, may be the preferable form of folic acid in some individuals, as long as we consider the adequacy of other nutrients involved in this cycle, including vitamin B12, vitamin B6, riboflavin, and vitamin B2.

Pattern Recognition

These processes do not work in isolation. There is a symphonic connection, a web of interaction among these single gene polymorphisms and other characteristics that form a pattern. Recognition of these patterns falls into the realm of what is now called medical informatics or bioinformatics. Researchers are trying to understand how these patterns, these clusters, are associated with specific disease risks. They are not just looking for "the gene" that causes heart disease, "the gene" that causes diabetes, or "the gene" that causes Alzheimer's. Instead, they are looking for characteristic patterns at the genomic, proteomic, and metabolomic levels that give rise to the relative risk or susceptibility factors for those disorders.

With regard to MTHFR specifically, scientists are learning that it is more than just folic acid or 5-methyltetrahydrofolate or 5-formyltetrahydrofolate. It is also riboflavin, vitamin B12, vitamin B6, and betaine, or other methyl donors in the diet. This is the topic of discussion in an article in the *American Journal of Clinical Nutrition,* titled "Impaired Functioning of Thermolabile Methylenetetrahydrofolate Reductase is dependent on Riboflavin Status: Implications for Riboflavin Requirements."[4]

A Functional Medicine View of the Clinical Laboratory

In looking at the clinical laboratory from a functional medicine perspective, we might want to know what the MTHFR genotype is. It might relate to cardiovascular risk, stroke risk, dementia risk, or depression risk. We would then want to look, in therapy, not just at folic acid as a single nutrient. We would look at the forms of folic acid, 5-methyltetrahydrofolate and companion complex nutrients such as riboflavin, pyridoxine, and cobalamin, vitamin B12, that work together to arrive at improved function in that metabolic series of events, the metabolome that controls the phenotype

Oxidative stress is another example. This is another area of considerable interest and controversy. How do you measure oxidative stress? If you could measure it, what value would it have in clinical management?

If you look at a strict pathology textbook and examine the clinical laboratory from a pathological perspective, there is no test on a standard blood screen that directly measures tissue oxidative injury. Instead, it is a functional state of the system that gives rise to the risk of a variety of phenotypes we would call different diseases. From a strict pathological perspective, we might say this is uninteresting.

From a functional perspective that understands the complex chronic origin of disease and the symptoms that arise from it, however, oxidative stress analysis may be very important. Therefore, examining the reduction/oxidation (redox) capability of the cell may be a cornerstone in understanding more about the metabolome and the functional status of that patient.

Assessing Oxidative Stress

There are many ways to evaluate oxidative states of cellular systems, one of which is to look at the injury that has occurred to the system. For instance, looking at oxidized lipids, the thiobarbituric acid test, or lipid peroxide test, measures the debris, or what we might call the embers that occur as a consequence of oxidative injury to fatty acids. Oxidized fatty acids breakdown and release malondialdehyde which then reacts with a dye in the laboratory test to give rise to a colorant that can be measured fluormetrically or colorimetrically.

The degree of lipid peroxidation can then be compared to a standard. Food chemists use this test to measure the amount of rancid oils in food, and we can look at biological rancidification in our own blood as an example of lipid peroxidation. This test is a downstream extracellular marker for injury caused by peroxidation, because normally, peroxides are measured either in plasma or urine.

Another way to measure injury is by using extracellular or intracellular tests for oxidative injury of DNA. In previous issues if *FMU*, we have described this test, called the 8-hydroxydeoxyguanosine test, or 8OHDG. It measures the amount of DNA damaged by peroxidative or oxidative injury. This 8OHDG test can be performed as an intracellular test by using lymphocyte DNA to evaluate the amount of intracellular oxidative damage to DNA that has occurred. An extracellular test also exists in which 8OHDG is measured directly in blood or urine.

According to recent studies about 50 percent of the 8OHDG levels measured in the blood are present as a consequence of what is going on in the nervous system. What we are looking at are those highly oxygen-exposed tissues and organs of the body that have been undergoing DNA damage, and presumably damage to other proteins or lipids. We can also look at protein carbonyls as a measurement of protein oxidation, or we can look at the damaged lipids by lipid peroxidation.

Examining Oxidative Status by Redox Quotient

Let's examine how we would evaluate oxidative status by looking not at what is damaged but at the state of redox, the reduction/oxidation quotient. A number of tests have the ability to look at how resistive the cell matrix is to an oxidative stress. You add a specific oxidative stress factor like T-butyl-hydroperoxide to a blood sample and examine how this sample can resist that oxidative stress. This is similar to the ORAC test, the oxygen-reducing absorbance capacity, which actually measures the resistance of a cellular matrix or plasma to an oxidative stressful event.

Presumably, we are looking at antioxidant reserve in this particular test, or what I call redox buffering. Reduction/oxidation can be buffered, just as pH can be buffered, or electrolytes can be buffered by the presence of adequate redox-active substances in the cell or extracellular matrix.

Why Worry about Oxidative Injury?

There are many ways to examine the tendency toward or presence of oxidative/reductive injury. But why should we care? Let me give you one example. Individuals who have major vascular, pulmonary, or inflammatory events have elevated levels of lipid peroxides, 8OHDG, and other markers of cellular injury as a consequence of oxidative events. Therefore, if we want to look at a tendency toward redox imbalance associated with these tissue pathologies, we might want to measure oxidized LDLs, lipids, and DNA to determine the state of affairs relative to redox in the cells.

One study that evaluated this tendency clinically looked at the effects of soy foods on blood lipids. The investigators intervened with some soy foods that were high in isoflavones and some that were low in isoflavones. Genistein and daidzein, for example, are known to have effects on hormone regulation and antioxidant redox status. The researchers assigned individuals randomly to high- or low-isoflavone soy food diets. They then examined blood lipids, oxidized LDL, homocysteine, and blood pressure. The objective was to determine the effect of these dietary interventions on the expression patterns in genes in average people that gave rise to either increased or reduced risk of, in this case, vascular disease.

Measuring Isoflavones and Cardiovascular Risk Factors

The researchers in this study found that when individuals consumed the high-isoflavone diet (about 73 mg of total isoflavones daily by substituting soy foods for animal foods), all cardiovascular risk factors declined. This positive effect was in contrast to the low isoflavone-containing diet, which contained about 10 mg of isoflavones daily, a very low level. The 73 mg-per-day isoflavone diet, 50 grams of soy protein per day, resulted in lowering of oxidized LDL, lowering of lipid peroxidation as a measurement of oxidative stress, lowering of total homocysteine, and lowering of blood pressure, as well as lowering of total cholesterol. In this example we use a test to evaluate functional changes that occur as a consequence of a dietary intervention. It shows that one can manipulate functionality with such an intervention. This study appeared in the *American Journal of Clinical Nutrition.*[5]

These are two examples of the use of the functional laboratory in determining the trajectory toward disease and contributions that may be associated with chronic symptoms in these complex disease patterns

A third example of the benefit of clinical laboratory measurements in functional medicine is in the area of detoxification, or biotransformation. Researchers are finding a number of specific nutrients in food that influence the expression patterns of the genes associated with phase I and phase II detoxification. The

benefits of these phytonutrients, or plant-derived nutrients, are directed at the phase I mixed-function oxidase enzymes, or the cytochrome P450s, or CYPs as they are often abbreviated. They extend to phase II conjugation enzymes that perform a variety of conjugation reactions, glucuronidation, sulfation, methylation, glutathione conjugation, and amino acid conjugation.

These pathways have to be in balance for proper detoxification of endogenous and exogenous substances so they can be eliminated from the body through the bile or urine. Specific nutrients found in specific foods can influence the expression patterns of both phase I and phase II enzymes

One example of the benefits of phytonutrients is the use of various cruciferous vegetables to upregulate phase II detoxification enzymes and produce what is called bifunctional modulation of detoxification. Bifunctional modulators lower the activity of upregulated or an overly activated phase I enzymes. Highly activated phase I enzymes, CYPs, can produce too much of what are called biotransformed intermediates, which may be more hazardous than the substances from which they were derived.

The biotransformed intermediates that come about through upregulation of specific isoforms of cytochrome P450 may, in fact, have more injurious effects upon cells than the substances the body was trying to detoxify. That biotransformed intermediate has to be further detoxified by a phase II conjugative enzyme to form a balanced process of phase I and phase II, delivering this as a system through the phase I and phase II transformational steps.

Bifunctional modulators are specific phytonutrients that facilitate that balance. They lower the excessive activation of specific phase I enzymes while upregulating the expression of requisite phase II detoxification enzymes. The result is improved detoxification and balance, lowering the steady-state concentrations of potentially injurious biotransformed intermediates.

Phytonutrients and the Wisdom of Nature
This is an interesting concept. It appears that Mother Nature had infinite wisdom and put into foods specific phytonutrients that interact with our genes to regulate the production of the proteins that control detoxification. In doing so, she made sure those phytonutrients would help produce a safe response by lowering the upregulation of phase I and regulating phase II detoxification.

Researchers have recently discovered that some of the many substances that do this are the glucosinolates in watercress and garden cress. Animal studies have shown these substances improve the detoxification of serious carcinogens and lower the potential risk for formation of neoplastic lesions when the animals consumed high levels of the glucosinolates found in garden cress or watercress. I refer to an article in *Carcinogenesis*[6]

Evaluating Bifunctional Modulators
Not only watercress and glucosinolates, but also a number of other phytonutrients influence expression patterns as bifunctional modulators. One thing clinical laboratories can now do from a functional medicine perspective is to evaluate genetic uniqueness of the phase I enzyme system and phase II detoxification enzymes. One can look at individuals who may be upregulated in phase I, or perhaps downregulated in their phase II detoxification, in which an imbalance has been produced and they have what is called imbalanced detoxification status.

The laboratory can help us measure the pharmacogenomic relationships of individuals to their environment. This gets us beyond "one size fits all" mentality that simply assumes no matter what you give a person, he or she will respond the same as everyone else. We know that is not true of pharmaceutical compounds. We also recognize it is not true in regard to environmental exposures. Each individual is unique in regard to detoxification based on genetic and nutritional intervention relationships.

Understanding Pharmacogenomic Relationships

The functional medicine laboratory can be of significant value in understanding the pharmacogenomic relationships of the detox profiles to various exposures. By intervening with specific nutrients known for their bifunctional modulation capability, a practitioner can design an individualized program for a patient to minimize the risk or improve his or her first-pass detoxification of specific substances. This is another application of the clinical laboratory in functional medicine

A variety of complex disorders have received considerable attention recently in regard to their evaluation from a clinical laboratory perspective. One is autistic spectrum disorders. We increasingly recognize that autism is another condition associated with the interaction between genes and environment. A series of papers recently submitted to the *Lancet* discuss Dr. Andrew Wakefield and his seminal paper looking at ileal nodular hyperplasia in association with autism, and its connection to vaccinations for measles, mumps, and rubella (MMRs).

Dr. Wakefield has received a tremendous amount of criticism. Some individuals have attempted to defrock him as a pediatric gastroenterologist of academic stature. His data, as controversial as they are, stand as an important point in our understanding of these complex disorders. Not every child with autism has had an adverse response to immunizations.

Dr. Wakefield's work reminds us that each of us has unique genetic responses associated through the lymphatic and immune systems that ultimately create different phenotypic outcome in expression patterns. Remember that the gut, the liver, and the white cells in the brain glia are interconnected through similar signaling systems. If you are interested in the autism/bowel inflammation/measles connection, you may want to look at the *Lancet* article I referred to for a discussion of MMR vaccinations and ileal nodular hyperplasia to assess the debate and its differing sides.[7] Although I do not take a side in this debate, I think Dr. Wakefield's contribution opens our eyes to the complex nature of disorders such as the autism spectrum.

Autism and the Clinical Laboratory

A review paper by Drs. Bernard Rimland and Woody McGinnis, titled "Vaccines and Autism," reviews this topic in detail.[8] The article is a scholarly work of significant magnitude, containing more than 150 references. You can order a reprint from the Autism Research Institute, 4182 Adams Ave., San Diego, CA 92116. That address will appear on this month's Summary Cards.

Autism spectrum disorders, the gut/brain connection, leads us into trying to understand, from the clinical laboratory perspective, where the specific problem may lie in that child. We know that different effects may be seen in different children. In the lab we look for recognizable patterns of specific metabolites within the metabolome that may reflect different proteomic and genomic expression patterns. These patterns then may be modifiable through intervention with specific dietary, detoxification, or complex psychosocial support programs. In other words, we can look for an integrated approach to give each child

the optimal opportunity for addressing possible underlying problems of immunity, toxicity, and metabolism. This approach differs from simple diagnosis and management of symptoms. We are trying to determine the underlying cause

This same theme applies to malignancy. Cancer is a disorder of major proportion, an understanding of the origin of which is still emerging. A recent review paper in the *Journal of the National Cancer Institute* discussed the prevalence of cancers in males from 1950 to 1998, and the relationship of this prevalence to various socioeconomic patterns. In 1950, according to the authors, people of higher socioeconomic patterns had a higher incidence of cancer. In 1998, however, individuals of lower socioeconomic status had higher incidence of cancer. They suggest that people who are better informed and better able to afford different kinds of foods and environmental quality have been able to mobilize this information and lower their incidence of cancer over the past 40 years. In contrast, those who are less able and less willing to introduce and implement these concepts continue to have increased incidence of cancer.[9] Is cancer a condition that is modifiable by genes and environment? The unequivocal answer seems to be yes. Therefore, genomic patterning, proteomics, and metabolomics become part of the assessment of cancer risk as well.

That concludes side I of this month's edition. Let's move to Side II

INTERVIEW TRANSCRIPT

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In the 20-year history of Functional Medicine Update, we have never before dedicated an issue to evaluating the clinical laboratory in functional medicine and examining the tools it brings to answer complex questions. We are fortunate this month to have representatives of three major laboratories that provide services to functional medicine practitioners. First is Dr. Brad Rachman, Vice President of Clinical Innovation and Marketing at Great Smokies Diagnostic Laboratory. GSDL has taken a leadership position in predictive genomics.

Dr. Brad Rachman, Great Smokies Diagnostic Laboratory
JB: I would like to ask you, Dr. Rachman, to tell us how you see predictive genomics in relation to functional medicine. What doors is it opening for physicians in managing complex patients?
BR: Jeff, you have been quite an educational provocateur in this area. I can remember six or seven years ago, you introduced many of us to the concept of genomics as a potential predictor of risk. The novel concept was introduced at that time that perhaps not all genes determined direct fate, but rather gave us a preponderance toward the situation and a potential road map with which we could change the expression of a patient's health. Yet, with all of that great information and a new paradigm, the tools did not quite exist at that time for doctors to implement into clinical practice the concepts you were sharing.

Great Smokies has been fortunate to have a phenomenal brain trust that acted upon the huge database of information that has come out of the Human Genome Project, in creating usable clinical panels of SNPs (single nucleotide polymorphisms) that have been selected for major criteria. The steps are relevant to a particular clinical presentation, like cardiovascular risk, osteoporosis risk, or immunologic risk, of up- or downregulation or detoxification risk.

SNPs and The Clinical laboratory
SNPs are certainly prevalent, which means there is some degree of certainty that these are going to show up in a typical patient population at a rate anywhere from a few percent to upwards of 30 or 40 percent.

They are modifiable, a key factor that the literature has demonstrated. We have some ability either to directly modify the pathway by which the protein's expression is being changed or to provide some alternative pathway.

And finally, they are measurable. We have some phenotypic testing in the industry, and the tools will be available to doctors. They could measure whether the changes they were recommending to their patients, and their patients were implementing, were indeed changing the expression of that gene and, most importantly, bringing that patient closer to health.

Single Nucleotide Polymorphisms
JB: I want to review what you just said, because this information may not be very familiar to many of our listeners. You talked about the single nucleotide polymorphisms (SNPs) that relate to specific criteria of disease risk. They have to be understood and measurable. They have to be modifiable through some kind of an intervention program. They have to have a reasonable prevalence; and there has to be some way of evaluating in the phenotype of the individual or in their performance or function, the outcome of those expression patterns. Is that a reasonable summary of what you said?
BR: You said it much better than I did.

SNP Testing at Great Smokies
JB: As it relates to this type of testing, you have chosen certain panels in the area of osteoporosis, cardiovascular disease risk, immunologic risk, and the detox genomic approach. Why did you select those? What were the unique features of those gene panels that gave rise to their being the first entrees into the field?
BR: As our initial research led us in the direction of looking at those SNPs via those four specific criteria, it was clear that the literature had developed a greater comfort around them in being able to provide matches for all of them than in many others. The intent of our Genovations development group is

certainly not to stop with those four. We've embarked on a program to release an additional six or seven panels in key areas by the end of the year. These were the low-hanging fruit, though.

Predictive Genomic Evaluation
JB: A molecular geneticist would probably say a whole series of genes are related to bone formation and bone loss, and a whole series of genes are related to cardiovascular disease. They might say this is a yet-to-be fully explored area of information. How did you select the genes you chose, and what caused you to comfortable in doing this predictive genomic evaluation?
BR: That's a good point to discuss. I am painfully aware, as is the research team, that much data remain to be mined out of the Human Genome Project than we've been able to encapsulate into these individual profiles. We've put a stake in the sand, if you will, and grabbed those profiles that have the most substantial literature behind them relative to their impact for long-term health in those key areas of physiology that we were talking about.

By no means, however, do they represent what the panel might look like a year from now. In fact, as our development group was looking at the cardiovascular panel, which has nine or ten SNPs, we found that each of those SNPs might actually have several loci or locations across the genome that might be simultaneously measured. That number is twice as large as it was when the development group actually specified the initial panel. And, as we go to our release of this panel, just four short months after we were performing it for the first time, we have already begun to find additional SNPs. It's a growing field, and it's extremely exciting to be on the forefront of it.

We continue to test the viability of our model of looking at those SNPs that are prevalent, relevant, modifiable, and measurable. We have found, at least to date, that it has proved to be a good standard against which to measure the numerous articles we find every day. We're happy to be finding additional SNPs every day and bringing more clinical relevance to these profiles.

Clinical Relevance of Functional Genomics
JB: This is all very exciting, but what does it mean to the practitioner, in terms of helping him or her more successfully manage complex patients?
BR: Clinical application represents, I believe, the greatest win in the area of predictive and functional genomics. Most of the world is looking at the outcome of the Human Genome Project and trying to answer an age-old question. Is disease locked into the genes, or is it somehow locked into environmental influence?

In the last half of the last century, medicine and public health made great strides in appreciation for the environment, which would include a patient's lifestyle, diet, and specific nutrient intake. Many people are looking at the results of the Human Genome Project with a bit of a jaundiced eye. They had hoped we would find the opposite, that almost every human disease has a definable locus or definable place on the genome where we could look and identify and perhaps eventually get into and genetically modify, and cure diseases once and for all.

A Complex Interaction of Genes and Environment
We're finding out, much to the chagrin of many researchers, that the answer isn't becoming simpler; it's becoming more complex. What we find now is that the end result of a patient's health expression is a complex interplay between genes and environment. What predictive genomics does is offer doctors who

are working with the complex patient a new set of tools. It gives them an entirely new perspective by which to expand their vision of a patient's genomic risk. It is enabling them to reach a greater understanding of the predecessors that led to the point at which the patient's current health expression began to exist. They can actually look at those antecedents as a series of programmable errors in the genome that might be expressed at this time.

It literally helps practitioners not only to narrow their diagnostic vision, but also to get very precise relative to therapeutics. They are able to tell a patient with a greater degree of certainty than ever before that some things are "nice to do" and others fall into a completely different category of "must do."

Focusing on Individual Patients

We all have a series of health recommendations we can make to patients. If patients did everything we recommended, they wouldn't have time for work or play; they would simply be taking pills, taking care of themselves, worrying about their sleep, worrying about their spiritual nature, and tending to all our recommendations. But not everything we recommend is appropriate for every individual. We were previously practicing medicine for the mean, looking at large epidemiological studies, and saying, for the average person, if you're lucky enough to fit in the center of the curve, this is going to be a nice thing for you to take.

The results of these predictive genomic tests actually help focus into a preventive plan for a patient, a plan for intervention well before the expression of disease or well before the expression of phenotype. We can arrive at a very narrow "must do" list related to higher risk factors for that patient. We can tell what preventive measures are important for him or her, measures to which that patient needs to adhere for the rest of his/her life.

As a result, we're hearing back from doctor clients who are implementing predictive genomics that their level of compliance is higher than they have ever seen before. Regarding patient instructions, lifestyle interventions, nutrition, and dietary changes, this form of testing is providing a better form of communication than they have ever had before.

The Cardiogenomic Panel

JB: Would you summarize for our listeners how they would collect a sample and utilize one of the panels GSDL has available? What information might they get back from the laboratory?

BR: The cardiogenomic panel is a good example. All of the panels evaluate DNA. DNA can come from a variety of sources. The panels can be performed using either blood or saliva. With a blood draw we're looking at the buffy coat, which is the resident material, the DNA. In using saliva, a lot can be done with a buccal wash, a simple mouth rinse procedure. The patient rinses some alcohol-based mouthwash in his or her mouth for two mornings in a row, and spits it back into a sterile collection tube.

We actually collect more DNA from a buckle rinse than we do off of the Buffy code, but because DNA is DNA, both are viable solutions for the collection of the DNA, and it really depends not only on doctor preference, but on compliance issues. Some doctors like to have the blood draw performed at the moment. They've introduced this concept to a patient to assure that it will be done. Others prefer, because of biohazard complications and OSHA regulations, not to do blood draws in their offices. Either is fine.

Looking at DNA in the Laboratory

The sample is sent to the laboratory. The DNA is remarkably stable. We perform analysis at the point that breaks down SNPs into functional areas. In the area of functional testing, we've all been introduced to panels of tests that collect analytes into functional groupings. In the cardiogenomic panel, we look at polymorphisms that fall into four basic categories: those that can affect or control the expression of cholesterol metabolism; the apoE polymorphism that you've educated us about for years; selectin; and cholesterol estertransferase protein. We're also looking at methylation defects as a second category and at the methylenetetrahydrofolate reductase regulation gene as a possible error in folate metabolism. This error can have possible sequelae a methylation defect, as an inborn defect. It is a very interesting area.

The third general classification of SNPs involves hypertension. We're looking at GNB3 and angiotensin receptor and angiotensin gene controlling areas that have predominant control over the mechanisms in the body for primary and essential hypertension.

Lastly, we look at the potential for coagulation defects as an error in the regulatory mechanisms for factor 2 and factor 5. Both of these have been well defined as clotting mechanisms. We find them particularly useful as demonstrating genes for accelerated risk, especially for women who are taking oral contraceptives. In fact they are useful for any individual for whom Michael Schmidt's blood/sludge theory might be a key factor in the etiology for atherogenesis.

Widening the Clinician’s View of the Patient
Together, these elements make up an overview of some of the key factors that lead not only to atherosclerosis, but also to hypertension and resulting stroke. They can give clinicians an ability to look at patients through a broader lens, if you will. They can look back into their health history, actually back all the way to their genes, and try to draw conclusions effectively for the health intervention and monitoring of these patients.

As with all of our tests, the description of each of these SNPs, as well as some possible considerations for intervention, is included in the report that goes to the clinician. Many clinicians find it helpful, since this is a new field, to receive reference material with the report so they can orient themselves on a regular basis and help orient their patients on the importance of these SNPs and the specific interventions that are appropriate.

Dr. Alexander Bralley, MetaMetrix Laboratory
JB: Thank you, Dr. Rachman.

The second laboratory representative with whom we are going to speak is Dr. Alexander Bralley, founder and president of MetaMetrix Laboratory, a long-standing friend and colleague, and one of the founding fathers of the functional medicine movement. Andy, perhaps you can tell us how MetaMetrix Laboratory sees the functional medicine doctor's needs from the clinical laboratory perspective.
AB: Thank you, Jeff. MetaMetrix has been specializing in metabolic and nutritional testing for about 20 years. We've helped design some of the basic tests that are used in the functional medicine approach. It's been an interesting experience over that time because, as you know, a lot of this material has not been taught in medical schools.

It has been a challenge for us to get the information out and relate to it in specific ways to the clinicians who use it. It has always been fascinating for me to see the difference in the types of testing that are done,

from the allopathic point of view versus the nutritional/functional medicine approach.

Studying Imbalances

To a great extent, our tests are not designed to diagnose a specific disease, as are many other tests that doctors have available to them. This fact represents a different approach, in and of itself. What we do is look at nutritional, metabolic, and toxicological imbalances that can relate to a variety of symptoms and disorders that the doctor sees in the office. With that information, the clinician can design a particular metabolic, nutritional, functional medicine approach to help treat the specific patient's needs.

This has evolved into an interesting concept over the years. When we first started the laboratory, we saw the need to begin to pair specific disease types to specific metabolic, nutritional profiles. For example, what does an autistic nutritional or metabolic profile look like? What is uniquely true about autistic metabolism that might relate to the tests we're picking up? Is there a pattern there? Are there specific patterns in CFS patients, fibromyalgia patients, or those with inflammatory bowel disease (IBD)? For years, we've looked for those types of markers and, in some cases, you do see them.

Studying the Patient’s Unique Genetic Makeup

More interestingly, as we have gained experience we have begun to realize that we're not really looking at a disease process itself. What we're looking at is the expression of the person's unique genetic makeup that takes a specific imbalance or set of imbalances and expresses them in unique ways.

For example, say we do a panel of tests of amino acids, organic acids, fatty acids, vitamins, minerals, toxic metals. Two people can have very similar imbalances in those measures and yet express them in entirely different ways. One person can be very depressed with IBD; another person can have a skin condition. Yet the imbalances can be very similar. We really can't diagnose a disease; we're really diagnosing specific metabolic nutritional imbalances and toxicological imbalances that can relate to a patient's symptoms.

Introducing Practitioners to Functional Laboratory Testing

JB: When a physician first comes to you as a beginner in practicing functional medicine, how do you instruct him or her? You have just written, with Dr. Richard Lord, a book titled Laboratory Evaluations in Molecular Medicine: Nutrients, Toxicants, and Cell Regulators. I highly recommend this book, whose topic is the use of the clinical laboratory and predictive testing in functional medicine. How do you capture this in helping doctors take the first step?

AB: That's a good question, and we're seeing more and more doctors become interested in this area. The book has been useful, because for years, our clients have been asking us where this is written down. Where can they study this more? There really has been no textbook available that talks about functional lab testing. The book is useful in that sense. It contains 1500 references from the medical literature to explain and clarify the different tests and how to use them clinically. It is a useful tool we can give to a doctor who calls and wants to use that type of testing.

Often, we recommend a few basic tests that provide a lot of useful information in a cost-effective way. There are three tests we usually recommend for a clinician to get started—the IgG food antibody assay, fasting plasma amino acid testing, and organic acid analysis. Those three panels provide a wealth of information with very practicable, useable, therapeutic advice to treat a variety of different symptoms.

Laboratory Evaluations in Molecular Medicine

JB: With regard to the book, tell us where it's available. I highly recommend it for anyone who wants to be a skilled practitioner in functional medicine.
AB: It's available on our website at metametrix.com, or they can call our office and order it at 800-221-4640.

Distilling Information from Tests
JB: Let me go back to the three tests you've recommended as the frontier tests, the foundation tests, the IgG test, amino acid analysis, and urinary organic acid analysis. Some individuals might feel you get so much information in these tests that you went from too little information with traditional pathology tests to an information glut and overload, and it's like trying to drink from a fire hose. How do you help doctors distill down the tremendous amount of information you derive from those three tests into clinically meaningful clusters?
AB: There are two basic ways. First of all, that's always been a challenge for us, since these are new tests and people have to learn new terminology and drag up their biochemistry from medical school. We provide interpretive guides with every test that give very specific recommendations, based on the test results. Food allergy testing is rather easy to deal with in terms of elimination, rotation of the foods that are elevating immunological response in terms of the IgG response.

In the amino acid analysis, we are primarily looking at deficiencies of the essential amino acids. We've actually developed an algorithm that recommends a specific essential amino acid formula based on the person's unique perceived needs from the test. That is a very easy way to utilize that test as a therapeutic tool.

Interpreting the Tests
The organic acid analysis is a bit more complicated. It looks at both nutritional metabolic imbalances and dysbiosis markers, things that appear in the urine that are being made from dysbiotic organisms growing in the gut and elsewhere that do show up in the urine and act as little metabolic monkey wrenches. We provide a very detailed interpretive guide containing therapeutic and supplement recommendations with that test.

In addition, we have doctorate-level consultants available here for free consultations. They spend most of their time talking with our clients, discussing patients, discussing test results, and helping them with the therapeutic approach to each patient. We try to offer a full range of services that make it easy for the clinician to utilize these tests and apply them in practice.

Third Party Reimbursement and Functional Testing
JB: You spoke about the difference between a diagnostic test that arrives at a specific ICD-9 diagnostic code and a prognostic test that evaluates the functional capability of the individual and the trajectory toward a disease, or functional status. We all know the bias in the healthcare reimbursement system over the last 30 years has certainly been on the diagnostic side, not on the prognostic side. How does a doctor handle this prognosis versus diagnosis dialectic?
AB: That's an interesting and challenging question, and I'm not sure I know the answer. A lot of the doctors we work with basically work on a cash practice business. They do provide detailed receipts. We also provide detailed receipts to send to insurance companies for reimbursement. In many cases, they do get reimbursed with proper diagnostic coding and CPT codes.

It's starting to happen. I think it's becoming a more and more acceptable approach as time goes on. The Director of Medicare here in Georgia has told us that even though they don't reimburse for tests they consider research and/or investigational, or screening, he views our tests as the wave of the future. He believes that Medicare will eventually have to reimburse for this type of testing because it's very cost-effective.

Common System Difficulties Observed in Functional Lab Testing
JB: When you look at the patients who have the most common clusters for which the laboratory is providing information to clinicians, are certain kinds of system difficulties more common in this kind of testing?
AB: The wide range of types of symptoms we are seeing is really remarkable. We see significant inborn errors of metabolism like Down syndrome, to chronic diseases, primarily where the modern medical system has failed to produce an effective treatment. We see all types of things, from mental/emotional disorders to skin conditions, IBD, joint problems, and fatigue and pain issues. The wide variety of different types of patients we deal with over time constantly amazes me.

Chronic versus Disease Management
JB: Would you say that in general, the laboratory provides services more in the chronic versus the acute disease management area? I assume that would be the case.
AB: In most cases, that's correct.

Functional Laboratory Applications in Chronic Disease Diagnosis
JB: From articles we've seen in publications like the New England Journal of Medicine and presentations we've had by experts at our International Symposium on Functional Medicine, it appears that chronic disease management is lagging far behind acute disease management in this country and throughout the world. MetaMetrix Laboratory is a pioneer in developing new approaches for recognizing the origin of mechanisms underlying, and hopefully the remediation of chronic illness.
AB: Yes. We've been very active in developing new technologies for measuring the various components we're interested in and constantly on the lookout in the medical literature for new things to be looking at and how they relate to chronic illness. Last year we started a joint venture with two physicians here in town to do molecular genetic testing and started a molecular medicine laboratory here at MetaMetrix.It's kind of a division of MetaMetrix.

It's interesting because we're now testing the entire spectrum of disease, from severe inborn errors of metabolism to the chronic illness model where we're looking at weaknesses in the genetic structure that simply take longer to express themselves. They're not expressing themselves in the first month of life, but they start to express themselves in combination with environmental factors as we age. We have the ability now to look at the entire spectrum of disease processes, from the very acute severe genetic disorders to the more difficult ones to deal with like why is a person getting sick and they don't know why.

Functional Medicine Testing Pioneer
JB: In the 20 years you've been involved with the clinical laboratory business, you have been a pioneer in opening up tools for doctors, not just to speak about functionality but actually to be able to measure it and do something about it. You have my strong support and appreciation for the work you've done. Your book will be listed on the back of the summary cards of this month's issue for those who want to follow up. I recommend it highly.

AB: I appreciate that, Jeff, and I'd also like to thank you. You were instrumental in getting me to start down this crazy, wonderful pathway way back when. I appreciate what you've been doing, too.

Darrell Hickcok, Doctor's Data

JB: In concluding our discussion of the clinical laboratory in functional medicine, our third representative is Darrell Hickcok, the president of Doctor's Data, a pioneering laboratory in functional medicine assessment. Darrell has been involved in this field for many years. Doctor's Data goes back more than 20 years, with its initial work in essential trace mineral assessment and toxic mineral evaluation. Welcome to FMU, Darrell. Tell us about the history of Doctor's Data and the development of the essential trace element evaluation and the toxic mineral evaluation.

DH: Thank you, Dr. Bland. Actually, we are celebrating our 30th year in 2002. In 1972, we started hair trace metal analysis, which was quite revolutionary for the time. The theory behind that was that we wanted to look at the exposure to and excretion of chronic levels of metals, as opposed to the acute levels that you would find in the blood. We have added many different matrixes to our testing menu since that time. We do urine analysis, which is primarily used for chelation and provocation testing. We measure whole blood RBC mineral levels, looking at circulating levels of metals and intracellular levels of metals. Our latest addition is the fecal metals test, which is a marker for oral exposure and biliary excretion of metals. This has proven to be quite popular for doctors working with autism. Those are the basic functions of our laboratory. We have since been moving into the microbiology/parasitology/amino acid market.

Provocation Testing

JB: You mentioned provocation testing, which is an important point of differentiation between clinical evaluation in functional medicine and typical pathology evaluation in the laboratory. Provocation testing involves challenging a patient with a specific substance and looking at the patient's response to that challenge. Examples of the difference are the oral glucose tolerance test compared to fasting blood glucose, or an exercise EKG versus a resting electrocardiogram. In provocation testing you place a demand on the system and look at its resilience. Would you tell us about provocation testing in relation to minerals?

DH: Absolutely. The basic technique involves the collection of a baseline urine sample, which is usually between a 6-hour and a 24-hour collection, depending on the agent to be used. This provides the baseline for differentiation. The next step involves administration of a provoking, challenging, or chelating agent, depending on the pharmacokinetics of the agent. It could be DMPS or DMSA. Even vitamin C has been used in fecal metal provocation. But primarily it's a urine test.The agent is administered at appropriate dosage and then a second urine collection is taken for a period of time that is based on the half-life of the chelating or provoking agent. Then the two are compared and the differentiation provides insight into the patient's metal exposure, body burden, and total load.

Heavy Metal Toxicity

JB: I know that Doctor's Data has been an important educator and leader in the field of heavy metal toxicity, not just mercury, but lead, cadmium, arsenic, and aluminum. Please share some of the experiences you've had over the 30 years, to illustrate the importance of the heavy metal toxicity problem.

DH: I believe environmental exposure to heavy metals has been increasing over the years—cadmium, lead, mercury, and even more esoteric metals that are being used. Today they have more of a limited occupational exposure, but an article published in Circulation linked levels of mercury to certain cardiovascular diseases, cardiomyopathies. Levels of mercury were thousands of times higher in patients

with this disorder compared to patients without it. More and more research is exposing the role of metals as underlying causes of disease, organ damage, and learning disorders. As the field grows and expands, I'm certain we'll see metals emerge as the cause of more and more problems.

Metals and Chronic Illness

JB: The number of samples you have processed at Doctor's Data and the information database you have are probably second to none in this area. That database probably encompasses hundreds of thousands of samples. Have certain patterns emerged that connect body burdens of minerals to chronic clinical conditions?

DH: We do have a very extensive database, and there are interesting patterns that develop. Most of the patterns we see tend to be geographical, in where the patient lives. It truly reflects the old idiom that you are what you eat. In Japan, for example, we see much higher levels of mercury than we do in the U.S. population, and coastal people in the U.S. have higher levels of mercury and arsenic than those who live in the Midwest or bread basket portions of the country.

A source of debate among our company scientists has been whether or not there should be a reference range that takes into account the dietary factors. That is, a higher level of mercury may be normal or more acceptable for a person in Japan than it would be for a person living in Iowa. It's a debate that goes on in traditional medicine and all aspects of medicine to try to determine what a true reference population is.

Types of Testing

JB: When a doctor first comes to your laboratory, do you have some basic guidelines for starting into this type of testing? It may be different from what that doctor is familiar with in the pathology area.

DH: We break down our testing business into three main areas: gastrointestinal testing, metal testing (the provocation), and testing for nutritional status. The type of testing depends on area of interest in which the doctor is focused.

For GI, we recommend starting with a comprehensive stool analysis. This is a three-day collection that looks at bacterial cultures, yeast cultures, and chemistry markers of significance to the gut, immunology markers, fat markers, and things of that nature. In the nutritional arena, we offer amino acid analysis, which is a comprehensive test that breaks down amino acids as they relate to conditions such as GI function. It ties back in to immunology, neurological markers, detoxification markers. At the end of that test, we provide a summary of conditions one might expect based on the results. We also offer liver function tests that are functional markers of xenobiotic exposure and excretion. We are working on developing a protein load test that's based on amino acids to determine a patient's ability to process protein.

Protein Load Test

JB: The protein load test would be a good example of a provocative challenge test, where you're looking at the reserves of protein-splitting enzymes and transport processes of amino acids across the GI, so you'd actually be looking at a whole range of functional aspects by the provocation technique. That's a very interesting idea.

DH: Absolutely. We've been working on this for some time and feel that we're very close to offering this test. We've been doing some internal studies and it's very exciting.

The Future of Laboratory Assessment

JB: How do you view the future of the clinical laboratory in functional assessment?
DH: As the industry develops, more and more markers are becoming available. As detection limits become lower and more precise, we can measure things that the technology of 10 or 20 years ago wouldn't allow us to measure. It's a broad horizon. There are lots of interesting technologies out there. We've been looking, again in reference to protein, at the genetic aspect.

We feel that genetic testing, in which you determine if a person has a number of known genetic defects, is a very interesting possibility. When you apply the environmental exposure, that expresses itself as protein. If you can measure those proteins, then you have a deeper understanding of what's going on with that patient than you do if you merely measure the gene defect.

Genomics, Proteomics
JB: That's really the difference between what might be called the genomics model and the proteomics model, which looks at how the genes are expressed into proteins and how those proteins ultimately function as enzymes or structural components of the cell, or signaling molecules.
DH: That's absolutely correct. It's possible for a person to have 2 of 13 genetic defects, but without the environmental influence, they wouldn't be expressed into the potential disease or condition.

Recommendation to Clinicians
JB: Congratulations to you and all of the Doctor's Data group on your 30th anniversary. It is quite an achievement to be a pioneer through these 30 years in the development of this field. I believe that doctors who are starting down the road into functional medicine would be wise to talk with your professional staff about how they would get started in this laboratory arena.
DH: Thank you very much

Cancer Diagnostics

Cancer risk analysis has moved us into a range of new medical informatics, using pattern recognition and cluster analysis to determine what specific analytes would be most useful in defining a patient's relative risk, the trajectory toward cancer, or proper treatment. That leads us into cancer diagnostics, tumor marker analysis, and the complex gene arrays in which perhaps we would evaluate the expression patterns of thousands of genes to see which were being expressed that might lead to malignancy.

We have not yet reached the point where this has emerged as a fully mature field, but the early stages indicate we are heading in the right direction. A review paper in a recent issue of *Clinical Chemistry* looked at cancer diagnostics from the tumor marker proteomic/genomic perspective.[10,11] In this review, the authors explain that cancer markers are easier said than done. They discuss the theory that we will one day be able to tease out specific clusters of genes that give rise to specific cancer incidence or risk. Right now we are amassing evaluations of an array of genes and their protein outcome, using specific density gradient methods or gel electrophoresis. We may soon also be able to use other yet-to-be-identified technologies to increase our understanding.

Early-Warning Cancer Diagnosis
This technology will lead to early-warning diagnosis, well before a palpable tumor or even a radio opaque

image appears on a scan. It will give us the ability to start early intervention, so it may be much easier to avert the progression of cancer than at the later stage. By early intervention, I mean at the single cell or early stage in cellular replication. It would involve a single cell or 100 cells, not 1000, or 10,000, or 100,000 cells. It is an exciting time using new technology for assessing metabolic shifts in the phenotype that occur as a consequence of interaction of the genes and the environment.

One important marker that is emerging concerns inflammation. The metastatic process, the angiogenic process associated with tumor replication, is associated with increased inflammatory response. That is why antiinflammatories are seen as possible tumor-prevention or chemotherapeutic agents.

A couple of papers appeared in the *Journal of the Cancer Institute* recently under the title "COX-2 in Cancer—A Player that's Defining the Rules."[12] Isoform- 2 of cyclooxygenase is the inducible form we have heard so much about recently in relation to inflammatory disorders like osteoarthritis. COX-2 is also upregulated in the inflammation associated with tumor metastasis and angiogenesis. Therefore, according to these authors, selective COX-2 inhibitors may play an important role in downregulating the expression of this inducible enzyme and reducing in the inflammatory process associated with metastatic proliferation.

This is an interesting chapter in our emerging understanding of the web of physiological variables associated with a specific disease. There has been a tendency in the past to think of the pathophysiology of a disease as a single entity. Now we are starting to see a web of interacting physiological variables from gene expression patterns.

Dietary Substances Containing COX-2-Inhibitors

A number of naturally occurring dietary substances have COX-2 selective inhibitory effects. Many of these substances are found in foods that have recognized for their "anti-cancer influence." Included are spices such as turmeric or curcumin-containing Indian spices. Rosemary, another spice that contains selective COX-2 inhibitors, has been used historically in Ayurvedic medicine for its anti-tumor effects.

A variety of soy isoflavones have similar effects. We are beginning to witness alteration of some of these processes associated with inflammation that do not just connect to inflammatory arthritis, but also to heart disease, metastatic disorders, and malignancy. This research again indicates the important role the clinical laboratory can play in understanding specific mechanisms of disease that cut across the individual diseases themselves.

We hope you found this month's topics of interest. We will speak with you again in November.

Bibliography

1 Kiberstis P. Roberts L. It's not just the genes. Science. 2002;296(5568):685.

2 Strohman R. Maneuvering in the complex path from genotype to phenotype. Science. 2002;296:701-703.

3 Rozen R. Methylenetetrahydrofolate reductase: a link between folate and riboflavin? Am J Clin Nutr.

2002;76:301-302.

4 McNulty H, Mckinley MC, Wilson B, et al. Impaired functioning of thermolabile methylenetetrahydrofolate reductase is dependent on riboflavin status: implications for riboflavin requirements. Am J Clin Nutr. 2002;76:436-441.

5 Jenkins DJ, Kendall CW, Jackson CJ, et al. Effects of high- and low-isoflavone soyfoods on blood lipids, oxidized LDL, homocysteine, and blood pressure in hyperlipidemic men and women. Am J Clin Nutr. 2002;76:365-372.

6 Kassie F, Rabot S, Uhl M, et al. Chemoprotective effects of garden cress (Lepidium sativum) and its constituents towards 2-amino-3-methyl-imidazol

[4,5-f]quinoline (IQ)-induced genotoxic effects and colonic preneoplastic lesions. Carcinogenesis. 2002;23(7):1155-1161.

7 Hendrickson BA, Turner JR. MMR vaccination, ileal lymphoid nodular hyperplasia, and pervasive developmental disorder. Lancet. 2002;359:2051-2052.

8 Rimland B, McGinnis W. Vaccines and autism. San Diego, CA: Autism Research Institute;2002.

9 Singh GK, Miller BA, Hankey BF, Feuer EJ, Pickle LW. Changing area socioeconomic patterns in U.S. cancer mortality, 1950-1998: Part I--all cancers among men. J Natl Cancer Inst. 2002;94(12):904-915.

10 Pritzker KP. Cancer biomarkers: easier said than done. Clin Chem. 2002;48(8):1147-1150.

11 Diamandis EP, Bruns DE. Cancer diagnostics: discovery and clinical applications--introduction. Clin Chem. 2002;48(8):1145-1146.

12 Hawk ET, Viner JL, Dannenberg A, DuBois RN. COX-2 in cancer--a player that's defining the rules. J Natl Cancer Inst. 2002;94(8):545-546.p>