September 2008 Issue | Randy Jirtle, PhD Department of Radiation Oncology

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Welcome to *Functional Medicine Update* for September 2008. I am going to title this issue "Personalized Medicine and the Epigenome." Hold onto your seat. This is going to be a very interesting discussion.

In this issue we will have one of the world's leading investigators in the area of nutritional epigenomics, Dr. Randy Jirtle, as our clinician/researcher of the month. I think you are going to be really pleased with the quality of the information that he shares with us. Before we get to Dr. Jirtle, I think it would useful to set a context.

There has been a lot of fanfare and hoopla surrounding the age of personalized medicine that we are presumably moving into as a consequence of the decoding of the human genome. But as we get more into this topic we recognize the story is not quite as simple as we may have thought it to be back in the age of Watson and Crick and others who were looking at the triplet code that gave rise to our genetic lineage encoded within DNA. There is more to this genome story than just the linear sequence of nucleic acids along the polynucleotide chains of DNA.

We now recognize that DNA chains are wrapped together, not only in the alpha helix, but also supercoiled and compacted into our genome. They are insulated and regulated in a very interesting geometric-almost artistic-way into the nucleosomes, where the nucleic acid material is bound up in a selective way with histone and nonhistone proteins that coat it. These proteins provide protection from the outside environment so that chemicals and radiation do not have ready access to our book of life. Locked deep in this vault, more secure than Fort Knox, is the encyclopedia that we call our genetic code.

Unlocking the Epigenome

Unlocking this code requires selective library card privileges. You can only check out one book at a time and read each book individually. Because every cell has the complete library, if we were reading all the books simultaneously, our liver would behave like our heart, our heart would behave like our stomach, and our stomach would behave like our skin. All would behave like one another and we would be a mess. We have to differentiate function within this complex array of tissues, and the way that happens through developmental biology is by silencing some of the messages (meaning putting "Don't Read" messages on them, and putting "Read Here" messages on select portions of the genome that are related to the functions of that specific tissue type or cell type). These messages are encoded in what is called the epigenome. The epigenome (or "above" the genome) is the regulatory regions of the genome that allow for specific access to this book of life-our library, our encyclopedia. What has emerged in the last 10 to 20 years is that

accessibility to this message that is locked into our genome is in part regulated by interaction with our environment. Environmental factors influence the regulation of the epigenome, which then, in turn, regulates accessibility to the read and don't read messages of our book of life, our encyclopedia.

Personalized medicine is emerging as a buzz word or a sound bite that I think relates to understanding function. It is more than just arming ourselves with the decoded human genome. It is also looking at how the epigenome allows access to these bits of information that are encoded within our genome so they can be expressed as messenger RNA and ultimately into protein. Through post-translation modification by phosphorylation, oxidation, or glycation, these proteins ultimately become functional proteins that then regulate structure and function of the cell, tissue, organ, organ system, and whole organism. This is a very different view of regulatory effects on function, and ultimately on health and disease, than previously held thinking that all answers would come from deciphering the human genome.

Regulatory Effects of Environment on the Epigenome

We are at the beginning of the story. What is beautiful about this beginning story of the epigenome and the regulatory effects that the environment plays is that there is a lot more plasticity and modifiability in outcome than we previously recognized. We can't change our genes easily in the absence of mutation or molecular excision by genetic biology-related functions (by carving out a portion of the genome and inserting a new portion with molecular biology). So we can't change the genes easily, but what can be changed much more readily is the epigenome, by modifying the environment. Stress reduction, regular exercise, lack of exposure to toxic chemicals, proper nutrition and micronutrient intake, and phytochemicals have all been found to modulate the structure and function of the epigenome. Such modulation allows access to certain regions of our genome that then get expressed or not expressed into the proteins that ultimately regulate our cellular function. It is a very interesting story that is unfolding. You are going to hear much more about that from Dr. Jirtle.

"The Genome Gets Personal" is the title of a recent paper in the *Journal of the American Medical Association*.[1] The authors of this article talk about the fact that we are looking much more seriously at gene loci that map against certain diseases, and we are understanding more now about single nucleotide polymorphisms (the variation of single-letter alphabet changes in our code of life and how they may relate to diseases). The next step is understanding expression because it is not enough just to know whether you have or don't have the specific genetic susceptibility or strength. What is more important to know is if it is expressed into the phenotype and alters function in such a way as to result in health or disease. When we get personal with the genome, we have to take into account not only single nucleotide polymorphisms (or SNPs), but also how they are influenced in their expression by the epigenome and whether these SNPs sit in regions of cellular function that play principal roles in regulating outcome in the phenotype.

This is more than just genetic screening. Another recent paper in the *Journal of the American Medical Association* talks about delivery of genomic medicine for common chronic adult diseases.[2] The authors looked at how many titles have been published in the literature in the last few years in this area. It is over 10,000 titles that break themselves down into looking at outcomes of genomic medicine, consumer information needs, delivery of genomic medicine, and barriers and challenges to integrating genomic medicine into the practice of health care. The majority of these articles talk about multi-loci influences on the expression of chronic disease (multi-loci meaning there are not single genes that control the expression of the major chronic diseases: heart disease, cancer, diabetes, arthritis).

Chronic diseases are not monozygotic types of situations. They are controlled and regulated by multiple genes working together as families (or what are called cassettes of genes) that ultimately take their messaging from an upstream boss-the reporter DNA, or the area of the genes that actually regulate their expression, so these are the regulatory regions of genes. And these regulatory regions control the expression of multiple genes upon certain environmental features and reside in a place within the genome that is quite unexpected: they reside in what used to be called the junk DNA.

Years ago, I suggested that the term "junk DNA" presumed that this massive amount of DNA material found in our genome which didn't seem to code for protein was just relics from a past history that had no functional impact on the individual. Another point I have made (based on many other authors' work) is that the major difference between the genome of a human and all other plants and animals is the amount of junk DNA. If we look just at the coding regions of our genome, we find that we are 98.9{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} homologous with the chimpanzee. (A lot too close for some peoples' comfort.) But the difference between the chimpanzee and humans relates to the amount of non-coding DNA (what used to be called the junk DNA). It has now been discovered that this is where many of the regulatory regions for genes reside, and so no longer should we be calling this junk DNA.

These regulatory regions of genes are controlled upstream by the exposure they have to the ability to be expressed through epigenomic messaging (what we call silencing or activation). The epigenome lies on top of the regulatory regions of genes. These regions then promote the expression of downstream cassettes of genes that ultimately regulate a whole series of functions at the organismic level. A chronic disease is not a breakdown of one step, it is a dysfunction of the network of genes that regulate complex function. This is why chronic disease has multiple symptoms, not just one, because we are influencing many functions.

The delivery of genomic medicine for common chronic adult diseases is going to have to tie itself very closely to better understanding how the environment influences the epigenome and ultimately the regulatory or promoter regions of genes. One of the groups looking at this very intensely is a group at Rosetta Informatics, a division of Merck. Dr. Eric Schadt is the director of this group in Seattle, and they have been-along with a number of other groups-investigating the role that these genome-wide functions have on correlation with chronic disease. The Wellcome Trust Consortium has put together now what is called the GWAS, the genome-wide association studies. These have been published and now there are data sets looking at 14,000 people with various diseases and 3000 controls. These diseases include things like schizophrenia, diabetes, Parkinson's disease, and heart disease. One can go in and probe or query this GWAS database to see where linkages between disease and genomic and proteomic and metabolomic structure and function might reside. Dr. Schadt and his colleagues at Rosetta say you get a signpost from DNA that says there is something there, but you need to look beyond the signpost at how these are actually expressed in the function of the individual at these levels of protein expression and ultimately the regulation of metabolism. I am quoting from a recent article that appeared in *Genome Technology* in May 2008.[3]

Out of this derives the question: what environmental factors influence the expression of genes and the epigenome? That leads us, obviously, to one fundamentally shared principle that, as far as I know, every human being that has lived more than a day has had some experience with, and that is eating (i.e. nutrition). Nutrition is an environmental factor that influences epigenomics and gene expression. That

leads us into the discussion of nutrigenomics.

How does this relate to applications of clinical medicine? There are many papers that are being authored on this topic. One of note was in *Pharmacogenomics* in 2007 by Dr. Kaput and his colleagues and was titled "Nutrigenomics: Concepts and Applications in Clinical Medicine."[4] Dr. Kaput is now at the USDA nutrigenomics lab at the University of Arkansas. The authors point out that the maintenance of health and prevention and treatment of chronic diseases are influenced by many naturally occurring substances in foods, both macro- and micronutrients, as well as phytochemicals. In addition to supplying the substrates for producing energy (that is the caloric macronutrients: protein, carbohydrate, and fat), a large number of dietary substances (small molecules) are known to be bioactive. They alter the regulation of biological processes, and either directly or indirectly, they affect the expression of genetic information, translating down through the epigenome into the regulatory regions of genes and ultimately into gene expression. Nutrients and other accessory bioactive substances in foods, therefore may produce different physiological phenotypes among individuals due to genetic variability from person to person. Because of this genetic variability, no one diet will be optimal for all people because food is really information. Food influences the regulation of complex functional integrity at the cellular level based upon the genetic uniqueness of the individual. This sounds very much like Roger Williams and Linus Pauling, going back to the 1940s.

Nutrigenomic concepts, research strategies, and clinical implementation are similar to and overlap those of another field in pharmaceutical medicine called phamacogenomics, which looks at the individual way that drugs are metabolized and utilized based on their genetic difference. Both fundamental to the treatment of disease and the maintenance of optimal health, so we are seeing a revolution in thinking about how to access, implement, and harness this genomic information.

Dr. Steven Zeisel, who is with the Nutrition Research Institute, Department of Nutrition, at the School of Public Health and Medicine at the University of North Carolina, has authored a paper that helps to understand how this might translate to clinical application. This paper appeared in the *American Journal of Clinical Nutrition* in 2007 and was titled "Nutrigenomics and Metabolomics will Change Clinical Nutrition and Public Health Practice: Insights from Studies on Dietary Requirements for Choline."[5] His group has been looking very significantly at one of the B-complex vitamins, choline (trimethylglycine), and how it influences cellular function as a co-factor or as a nutrigenomic modulator.

You might recall me mentioning this study in a previous edition of *Functional Medicine Update*. The authors found that metabolic syndrome, or insulin resistance, in part relates to individual nutrigenomic response to the nutrient, choline. Large variations in responses occur within populations as it relates to choline, far greater, probably, than we have acknowledged through the recommended dietary intake or the previous RDA levels. This biological diversity at the genomic level is consistent with what Roger Williams talked about with his concept of genetotropic disease back in 1949 in *The Lancet*. It is more consistent than the way that we have defined the RDAs and RDIs for substances based upon the amount required to prevent nutritional deficiency disorders.

Dietary Effects of Choline on Cell Signaling and Network Physiology
It would appear that even micronutrients have small implications on cellular physiology. In specific individuals, things like dietary choline might have larger effects on modulation of cell signaling and network physiology. Recall, if you would, that choline is a methyl transfer compound, that is, the transfer

of methyl groups. DNA methylation usually occurs at cytosine bases that are followed by a guanosine, the CpG islands within our DNA regulatory regions. These are islands that then can be methylated by methylating agents, and choline is one source of methyl groups.

In mammals, 60 to 90 percent of these CpG islands are methylated. When this modification occurs in the gene promoter region, expression is altered. We call this gene silencing. Gene silencing puts a paperclip on those genes and downregulates their expression. Increased methylation is usually associated with gene silencing or reduced gene expression because the methylated CpG islands attract capping proteins that hinder gene expression. These methylated CpG islands that have these capping proteins then induce gene expression in a different way.

As I said, DNA is wrapped in proteins that are tightly packed together and they prevent access to the promoter sequence of genes. Methylation and a companion process called acetylation, and perhaps also biotinylation, of these histone proteins can either cause them to be silenced (as with methylation) or activated (like putting sticky notes on them as in the case of acetylation), creating channels through which transcription factors can pass and activate gene promoters. Transcription factors can be things like nuclear factor kappa B, which upregulates the cassette of genes that are associated with inflammation.

If you produce more access to the gene messages of inflammation, a lower signal might induce a higher inflammatory response. You might want to silence those messages by reducing NFκB signaling as a transcription factor while you are upregulating tissue repair processes and the cassette of genes that are related to those effects. These are found to be, in part, related to nutritional status because epigenomics of methylation is tied to the availability of active methyl groups through S-adenosylmethionine, of which a precursor (or methyl contributor), is choline. So by silencing specific labile genes (or promoter regions of genes) that are associated with the expression of dysfunction, you can then silence those genes by improved methylation pattern.

This is nutrition in the genomics era, and we can tie that together with things that relate to the benefit of specific diets. Why do certain historical diets seem to be associated with improved health and health outcomes? Dr. Jose Ordovas and his colleagues at the Tufts University Medical School Department of Nutrition and the Human Nutrition Center on Aging have been looking at this for some time and published an article in *Molecular Nutrition and Food Research* in 2007.[6] In this article, they talk about how this epigenomic modulation by diet can influence cell signaling associated with insulin sensitivity and inflammation. This may account for why the Mediterranean diet, with its complex array of phytochemicals that help to modulate gene expression patterns by epigenomic patterning, can induce or produce a better clinical outcome relative to insulin stability.

I think what we are starting to recognize is that diet is much more than just the prevention of deficiency disease. Diet is also functionally contributing specific markers for expression of messages that regulate the phenotype through the genome. Can you do preemptive nutrition of a proinflammatory state by using this information? That is what Dr. Philip Gillies talks about in a paper in *Nutrition Reviews* in 2007. --He says that nutrigenomics can provide nutrition sciences with a molecular basis for positioning nutritional bioactives, (functional foods, medical foods, and designer diets) to preemptively offset chronic disease.[7] This preemptive model of nutrition consists of multiple interdependent parts, not a single pathway, but rather a web-like network that can be simplified into principal component axes. The axes that are most important-what I often call metabolic acupunctures points-are the nodes of the network.

Placing a small amount of nutritional input at these nodes can have a large effect on function and can regulate the expression of cassettes of genes in such a way as to prevent states of dysfunction that we associate with chronic disease. As an example, omega-3 fatty acids are known to be epigenetic and nutrigenomic modulators that influence gene expression and functions at the protein level that are associated with inflammation and insulin resistance. Increasing, then, your omega-3 fatty acids downregulate the expression of those processes and upregulates signaling associated with proper insulin management.

This leads us to the view that genes are not our destiny. What we need to look at is incorporating molecular medicine into clinical practice so we can modulate expression of genes in such a way as to produce the most important positive outcome. In all of our books of life-encyclopedias that regulate our expression-are found stories of Greek tragedy. Some people may have more of those embedded in their book of life than others, but all of us have them somewhere-an oncogene, or a series of genes that encode under certain environmental exposures to increase relative risk to a certain warp of the network of physiology that we associate with a disease. The question is how to silence those messages while upregulating and normalizing the function of the stories in our book of life that are related to expression of good health. In terms of how you manipulate these functions, the disease state often requires a pretty hard-hitting intervention. In the subchronic state, (well before you get to extreme histopathology of the disease), it may be a much more mild effect that is required in order to modulate these functional states at the epigenomic and genomic level.

When we take a global snapshot of health care, the numbers of chronically ill people are escalating. We don't have enough medical personnel and hospital facilities to handle all these people when they get to the stage of being in an acute disease state and people are receiving inadequate medical intervention. How do we manage that on the other side (not on demand, but on supply side)? Demand is once a person is sick. Supply is preventing the number of people who are going to need services. Medical practice patterns that are designed to provide quick and effective amelioration of signs and symptoms are frequently not an enduring solution to many health afflictions in the chronic disease state. The drugs that we employ in a chronic disease management program are often those that block certain signaling processes related to signs and symptoms, but don't correct the underlying problem that relates to the ultimate histopathology.

Unfolding evidence appears to support a genetic predisposition model for health and illness rather than this fatalistic predestination construct. These concepts of susceptibility are modifiable through epigenetic and environmental factors that have enormous potential to influence clinical outcomes. By understanding and applying fundamental clinical principles related to these emerging fields of molecular medicine, nutrigenomics, and the exposure to environmental factors, physicians of the future are going to be empowered to address causality of affliction and achieve sustained reduction in the incidence of more severe chronic disease.

At this point, I am paraphrasing a wonderful article that is titled "Our Genes are Not our Destiny: Incorporating Molecular Medicine into Clinical Practice" by Steven Genuis, which was published in the *Journal of Evaluation in Clinical Practice*.[8] We can nutritionally and environmentally modulate the disorders of aging by focusing our attention on genomic stability and epigenetic signaling. John Mathers, from the Human Nutrition Research Center at the School of Clinical Medical Sciences, University of Newcastle in Australia authored a nice article on this topic in the journal *Mechanisms of Aging and Development* in which he pointed out that dietary factors have a profound effect on many aspects of

health, including aging.[9] And now we start to recognize that the way they do this-I'm talking about biological aging now-is partly through interactions with the genome, which result in altered gene expression.

Damage to genomic integrity that we call genomic instability is associated with virtually every disorder of aging, and nutrition plays a role in maintenance of genomic stability. As advances in the application of high-throughput genomic technologies in nutritional research evolve (the so-called nutrigenomic revolution), we are starting to witness a new approach to understanding the molecular mechanisms by which nutrition affects aging, well beyond what most of us learned in school (about the prevention of scurvy, beriberi, pellagra, xerophthalmia, and rickets). We are starting to see that epigenetic-modulated changes in gene expression are also extraordinarily important and occur throughout the lifespan. These changes may be more profound when occurring in the first phases of conception in utero, but actually can occur by altered methylation, phosphorylation, acetylation, and ubiquination pathways or processes throughout our whole life. What we have also learned is that our germ cells can be imprinted with our epigenomic tagging, or these methyl groups, or these acetyl groups. And those particular patterns, which may have been a result of what the individual was exposed to in their life, can then be passed on, in their germ cells, to their progeny, which means that you can have a very quick change in the phenotype of a population if you had a population change in their epigenome as a consequence of altered environment. This could be starvation. This could be hypernutrition. This could be environmental toxicity or radiation exposure. All of these can modulate and modify the epigenome in such a way as to set up an inheritance of that without even changing the code in your book of life.

Now I recognize that this sounds Lysenko-like (like we are talking about adaptation). In the 21st century Lysenko doesn't look quite as strange as he did in the 20th century, based upon the evolving understanding of the epigenome and how the environment can influence it. We are starting to recognize this might better explain how societal drift can occur in certain diseases very rapidly, like the question that has been asked so many times: why are we seeing such a rapid increase in autistic spectrum disorders in our population? Is it only as a consequence of better diagnosis? Or is it a consequence of some epigenomic changes that are occurring that regulate (in the developing nervous system of the child) different expression patterns into their phenotype?

These are very powerful questions that bring us back to looking, hard, at the variables that can influence epigenomic structure and function and how it then can pass on those traits through hereditability-into progeny, generations, and influence their function and ultimately public health-type drift. To examine this construct of genomic uniqueness as encoded through single-nucleotide polymorphisms and how that interfaces with epigenomics and ultimately a chronic disease, there may be no better clinical example than the concept of the methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms and their relationship to folic acid need and how that maps against homocysteine and ultimately into clinical incidence of atherosclerosis.

As it emerges, the story appears to be more complicated than we previously thought because this homocysteine construct doesn't appear to work unto itself in isolation as a single-point gene risk factor. Rather, it inter-relates with other genes in this cassette of genes that regulate a variety of functions, including apo E genes, cholesterol-ester transport genes, and fibrinogen genes. When you put all those together as a family of genes and look at polymorphisms within them and how folate needs related to MTHFR polymorphisms connect with fibrinogen polymorphisms, CEPT polymorphisms, and low HDL

and apo E levels. What you find is that that clusters of genes, not single genes, are found to correlate with carotid intima media thickness (or CIMT) in healthy men. Individuals who have certain polymorphisms are at high risk to atherosclerotic disease as a consequence of shift in their web of physiology that makes them have a higher need for things like folate, exercise, and factors that might increase their HDL. It is a complex array, rather than single genes, that controls regulated function. We are talking about programs rather than drugs because single molecules will probably not do the trick. Folate is a single molecule. It can have an impact by itself, but it has a greater impact when it is embedded in a program that sets a net over this whole system that improves functional expression. I am now quoting from a paper in *Clinical Genetics* in 2001 that talks about this connection among different genetic propensities and ultimately the expression of carotid intima media thickness and risk to heart disease.[10]

The complex story of epigenomics and the relationship with methylation patterns and how that ties with the folate cycle and how that ties with methylenetetrahydrofolate reductase polymorphisms can weave itself right down into transgenerational amplification of various factors (where diets and lifestyles have been changing so rapidly as to alter the function of these particular expression patterns). Let's say a diet that suddenly alters folate concentration or leads to an imbalance in nutrients that are required to modulate these processes can change methylation patterns of your epigenome. That changes the expression of cassettes of genes downstream, and now you get increased prevalence of certain disorders, like insulin resistance, metabolic syndrome, and cardiometabolic syndrome.

Let's look at obesity. Some think of obesity as being solely a consequence of too many calories. Recognized in developed nations for a decade, the obesity epidemic has become a worldwide phenomenon, and seems to influence all age groups, including women of child-bearing age. This has fueled concern that maybe-beyond the luxurious nature of calories-there is something that inter-relates dietary drifts with nutrigenomics. In a study using Agouti mice (which are mice that are genetically at risk to obesity and diabetes) the hypothesis that maternal obesity induces trangenerational amplification of obesity was tested. They took these animals that were genetically inbred to be obese, and they started changing the methylation patterns in their genome by supplementing them with folate and B-12, which increased methylation of their genome. What the researchers found is that the methyl donor supplementation prevented transgenerational amplification of obesity in these animals that were genetically at risk to obesity and diabetes.

This is a pretty remarkable suggestion: certainly excessive calories do play an important role in contributing to the pandemic of obesity, but it may also be other shifts in our physiology as a consequence of epigenomic changes amplify susceptibility to obesity and make it more of a clinical risk factor. I am now quoting from a recent paper in the *International Journal of Obesity* in 2008, which I think is very, very fascinating.[11] I want to emphasize again that this was an animal study (Agouti mice), but the finding was that by modulating their folate and methylation intake one can alter the transgenerational transfer of the risk to obesity.

Imprinted and more equal. The imprinting of genes (silencing certain characteristics in the epigenome and amplifying other characteristics) has become a major theme in our understanding of how the genomic message is ultimately expressed. A paper in the *American Scientist* by Randy Jirtle and his colleague, Jennifer Weidman, talks beautifully about why perfectly good copies of important genes are silenced and what impact this has on the phenotype.[12] Even more recently, Dr. Jirtle wrote a paper that appeared

in *Genome Research*.[13] This paper was on experimental identification of novel human imprinted genes and showed that some genes were much more susceptible to methylation and demethylation (putting methyl groups on and off) than other genes. These more labile genes are those related to control and regulation of environmental effects on the phenotype of the organism. Not all genes are equally influenced.

This all leads us to environmental epigenomics and disease susceptibility. A very good collaborative paper by Randy Jirtle and Michael Skinner at Washington State University appeared in *Nature Reviews and Genetics* in 2007.[14] In this paper, they showed that environmental toxicants at high levels can induce epigenomic changes that can then be passed on as heritable factors to the subsequent generations in animals, increasing disease susceptibility. If you think of our environment and what influence it is having on our health, this might play a role.

Lastly, I want to mention a paper in *Proceedings of the National Academy of Sciences* by Dr. Jirtle and his colleagues, Dale Wang and Dana Dolinoy on supplementing pregnant animals with folate and B12 and looking at what it does to counteract the bisphenol A (BPA)-induced DNA hypomethylation associated with cancer in early development. [15] The results suggest you might be able to "neutralize" some of the relative risk to environmental chemicals by proper epigenomic tagging through nutrition.

This is a revolution that we are going through-a revolution in thinking. The textbooks we have been learning from are incomplete and actually incorrect, relative to the new biology. You are going to hear from one of the world's leaders about where we are going, Dr. Randy Jirtle.

INTERVIEW TRANSCRIPT

Clinician/Researcher of the Month
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Here we are, once again, at that portion of Functional Medicine Update that we all look forward to each month. You never know exactly what new revelation you're going to have. We've had the opportunity to speak to people who are making the new medicine and the new biology a reality before our eyes. This month we are so privileged to have another member in that lineage. I think he will open the door for many of us as it relates to this discovery that looks at genetics from a perspective that is post-Mendelian and moves us away from genetic determinism into this, I guess you would call it, pluripotentiality in which the genes and the environment are speaking together to give rise to the outcome that we call our phenotype. Who better to introduce that topic to us than the person whose work has really brought this whole field to life, and that is Dr. Randy Jirtle from Duke University.

As a professor of radiation oncology, you might think his focus would be strictly on cancer and how radiation influences oncogenesis and metastasis, and certainly that is part of his work. But beyond that was this extraordinary discovery that started us down another path, to some extent, and that is the work

with the Agouti mouse that has received so much attention. His group supplemented pregnant Agouti mice, which we all know to be white fat mice, that we have all-in the field of obesity or in diabetes research-used at one time or another. These mice are predisposed to have hyperphasia and to get fat, diabetic, and have cancer and heart disease. When they supplemented these mice with folate and vitamin B12 at higher doses they found the offspring were animals that were a different color. So without changing the genes, they changed the expression of the genes to be a brown mottled color, and probably most interesting, they did not get obese and they did not get diabetes.

This kind of shook the whole traditional hallowed halls of inbred animals and genetic determinism and raised some very, very remarkable questions, questions that are above genes ("epi" being the prefix and meaning "above")-epigenetic questions. Dr. Jirtle's work with his group has started to pave the way for us to understand something that has been probably understood at a "rules of reasonableness" level, but not at a molecular biology or molecular genetics level until the birthing of this field.

Dr. Jirtle, such a privilege to have you on Functional Medicine Update. Help us understand how you came about doing the experiment with the Agouti mouse. Were you surprised at the outcome?

The Phenomenon of Genomic Imprinting

RJ: We got into this because I was really interested in the phenomenon of genomic imprinting, which is another class of genes (maybe we'll have some time to talk about later on) that are regulated epigenetically. But there weren't good ways to study the effects of environment and nutrition on that class of genes. This mouse model system was available and it had already been shown that nutritional supplements could change the phenotypes, or the colors, of these animals. What had not been shown, though, before our study in 2003 (this is work that was done by Robert Waterland when he was post-doc in the laboratory--he's now down at Baylor), was what was the mechanism by which this occurred? What he showed very clearly is that the color of the animals was changed completely because of degree of methylation and the little bit of DNA that is upstream of the Agouti gene, which is a transposable element, a retrovirus that jumped into that position in this one specific strand of mice. In the animals that were brown, methyl donor supplementation dramatically increased the methylation at that level, and basically the Agouti gene went back to being regulated normally. The animals were brown and no longer became obese, nor did they get diabetes or cancer. We knew before that there was this connection between what an offspring is exposed to, very early in utero or during pregnancy, and adult disease susceptibility. What this experiment really did was demonstrate what was the memory, or the glue, or the gravity that held those two different time points together and it is basically epigenetic changes.

JB: So for those who may not be quite as familiar with the terms epigenomics or epigenetics as others, can you kind of give us a quick summary/primer course on this concept of promoter regions of genes and histone proteins and the genome and how it gets signaled to be expressed?

RJ: First of all, I like to use analogies because it is easier for me to understand. I really think of the genome as being comparable to the hardware of a computer. I think of the epigenome (which, as you said, means above the genome) as being comparable to the software that tells the computer when, where, and how to work. So epigenetic changes are basically changes that can be inherited during cell division that alter the function of these genes without changing the hardware, or the DNA sequences. So we are talking, really, about software programming of the genome. And there are two major, sort of, epigenetic-type phenomena that control expression of genes. There is obviously transcription factors that bind to

promoter regions, which are the bits of DNA that are just upstream of the genes and telling the genes when, where, and how to work. But on top of that, also, is sort of a memory system that, in effect, either allows a gene to be functional and regulated, or to totally be non-functional. Those phenomena are DNA methylation and histone marks, or histone codes, that when they are, let's say, methylated, for example, at the DNA, usually that means that the chromatin is condensed down. So, the transcription factors are the little fingers that go into those grooves of the DNA, and the gene is, in effect, turned off.

Rationalizing the Work of Mendel

JB: So, when we look at this concept that comes from Mendel, the dominant and recessive patterns of inheritance, which all of us kind of got exposed to probably early in grade school-that we had to reproduce pedigrees and look at various ways that dominance would kind of proceed over that of recessive traits-how do we rationalize these two things together? It seems like it almost throws into controversy the construct back to Lysenkoism?

RJ: It only does if there is some ability for these marks to be transferred from generation to generation. When I said heritable, what I meant was heritable during cell division. But there is also evidence that these epigenetic marks are not always completely reset. I like to think of this like when I was a kid and played with these little etch-a-sketches. They were the plastic that went over the wax and you'd write on them and then you'd flip them up and all the marks are erased and you can start all over again. That is what happens in most situations, but occasionally at some locations that doesn't happen. So in effect, what you now have is a legacy from the previous generation, and that legacy has now been created by the environment. So there is the possibility at certain genetic locations of being able to transfer this epigenetic information from generation to generation. If you are not continuously exposed to this, this should probably start dying out and being erased completely, but it is a way in which you can pass information to another generation about the environment that your parents and grandparents were exposed to. It is an adaptive mechanism.

JB: It sounds like, in terms of timeline, that this adaptation can occur very rapidly in contrast to natural selection by mutation, which we think of as smoothing over millions of years.

RJ: Right. There is a time compression component, which is something that would be very important because, for example, if you are in the middle of a famine it would be advantageous to be able to store energy more readily, and probably to be of smaller stature. We always focus on the negative aspects of this-in other words, disease susceptibility-but you wouldn't have these types of problems (i.e. diseases) if, for example, probably you didn't have a mismatch between the environment that you perceive you are going to be in versus what you find yourself in. This generation, now, or in the last couple of generations, might have been some of the only time in the history of humans where you can get a lot of calories, basically, in your diet, without expending virtually any energy. You can see that if you'd been in centuries and centuries of, say, very low nutritional status, that all of a sudden now you can get a lot of energy where a person could, if they were very efficient in storing energy, etc., you could become obese.

JB: Like Neel's "thrifty gene" concept, and like the Pima Indians that may have genetic selection for being more thrifty and therefore they are the yellow canaries?

RJ: Correct. This adaptive system, now, can also be pushed too far. So if you are exposed to environmental toxicants, for example, that we maybe never were ever exposed to, you can see now you

also have a system that you can push too far, to the point where it literally almost becomes broken. So it is not always just the mismatch that is the problem, sometimes you can really push it too far and create problems by the environment that you are being exposed to.

Epigenomics and Disease Susceptibility

JB: I think that review that you and Michael Skinner at Washington State University recently co-authored in Nature Genetics is absolutely spectacular, titled "Environmental Epigenomics and Disease Susceptibility," because it certainly raises the question-well it actually comes from some work that you published previously in PNAS that was related to bisphenol A, which seems to be in the news recently, and DNA hypomethylation- how an environmental toxicant can influence methylation patterns and epigenetics? Maybe you could help us understand how this environmental connection fits into this whole scheme of changing disease patterns?

Work with Agouti Mice and Bisphenol A

RJ: Yes. Now you have a system that is adaptive and involves epigenetic changes in programming. This is work done by Dana Dolinoy, who is now going up to the University of Michigan and starting her own laboratory. These are really (I think) some very interesting experiments because she came from a toxicological background. She was interested in these compounds that are referred to as endocrine disruptors, so they are estrogenic compounds and they mimic estrogens. They are also referred to, sometimes, as non-genotoxic carcinogens. In other words, they don't cause mutations, but yet in some animal models you get cancer formed from these compounds, and in this situation I think there is even evidence that you can get transmission of that from generation to generation.

If you think of the Agouti mouse model that we use, it is almost the biosensor for determining whether environmental compounds are capable of altering the epigenome and whether they primarily cause decreased methylation or increased methylation. So when we use the methyl donors-from food, basically (food supplementation)-we found increased methylation and we shifted the distribution of the offspring's coat color, let's say, to brown. Normally, it sort of sits right in the middle, more sort of right at the Agouti (mottled-type animals are the most prevalent).

So then we asked the question whether or not something like bisphenol A, which is a relatively strong estrogenic compound, can alter the epigenome, and Dana clearly showed that it caused hypomethylation. So now in this strain of animals, those animals become obese and would have a higher probability of developing diabetes and cancer in the future. So again, this is the first time when an environmental toxicant, or non-genotoxic agent has been looked at for its ability to alter the epigenome, and in this situation (in this mouse strain) we found that it caused hypomethylation, which is deleterious.

JB: As I recall, in that work, in at least one of the papers that Dana Dolinoy and your group published, you also found that nutrient supplementation of the mother helped to counteract the bisphenol A-induced hypomethylation. Do I recall correctly?

RJ: Yes, it almost gives you goosebumps, doesn't it?

JB: It does.

RJ: I mean people have already said-a long, long, long time ago-that food is medicine. So what we did is-

again, it sounds simple-we looked at the distributions of coat colors of animals' offspring that were exposed, for example, to methyl donor supplements, or genistein, which is a weak phytoestrogen, a compound which we also demonstrated increased methylation. It has to do it through a different mechanism than, let's say, folic acid because just genistein does not have a methyl group on in so it cannot donate methyl groups, but it is somehow enhancing the probability that those regions will be methylated. And so then we asked the very simple question: if the mother is exposed to a toxicant like bisphenol A in their diet, can you alter the effect of bisphenol A on the epigenome by supplementing the mother's diet with either methyl donors like folic acid, choline, betaine, or vitamin B12, or genistein? What we showed is that indeed, with these concentrations, we could completely block the negative effect of bisphenol A on the epigenome in the offspring.

JB: That is goosebumps. Quite honestly, it is like moving up to another platform for future studies, isn't it? I recall, many years ago, Bruce Ames had a cover article in Science magazine that was titled "Dietary Carcinogens and Anticarcinogens," where he was talking about the fact that there are substances within foods that can cause cancer, like aflatoxins, and then there are those substances in foods that may prevent cancer. This is prior to the epigenome discoveries that you've made, so it may help explain some of these anticarcinogen properties from a mechanistic level.

RJ: I think it can, but I want to make a point real clear, here. I mean with the levels that we were using we were able to negate, in effect, the negative effects of bisphenol A. That doesn't mean that all levels that people potentially are exposed to (to environmental toxicants and pollutants) could be counteracted by supplements in our diet because it could be very possible that you could totally overwhelm the system, also. But at the levels we were looking at, we were able to block completely the negative effect.

JB: I want to go back, if I could, just for a second. You alluded to the fact earlier that methylation is one of these important processes to silence gene expression in certain loci within the genome and it has to do with methylation of these CpG islands and the promoter regions of genes, but you also said there are other mechanisms (phosphorylation is one) that presumably come through kinase activities, and now we recognize that kinases are regulated in part by the presence of various phytochemicals in the diet. So it sounds like we have a very complex network of potential interacting variables that regulate the epigenome's kind of control mechanisms.

RJ: Yes, that's true. As I said, the histone code is much, much more complicated than DNA methylation and we primarily study DNA methylation because, in general, they tend to go hand and hand. If you have negative marks, in effect, on the histones (like deacetylation and that kind of thing, where you would have condensation of the chromatin), you usually have also hypermethylation. But it is very, very complex, and the two are working hand in hand. Right now, as I said, people are working and whole labs are working in one group (like histones, basically) and other groups of people tend to be working more with DNA methylation. Our lab tends to work more with DNA methylation because frankly it is a little bit easier to work with because you are still working with DNA.

JB: So if we were to address the question that people have raised, which I think is a question, probably, without a complete answer at this point: if undermethylation is undesirable (and that means that you are not getting proper nutrition of these folate-cycle-supportive nutrients that regulate S-adenosylmethionine production) then what about too much B12 and folate and hypermethylation? Where are we on that and how does that relate to the homocysteine story? I'm sure all of this has come up in your discussions?

RJ: Again, I'm not too much of a biochemist. I always work in analogies. I always use the analogy that a glass of wine might be good for your cardiovascular system, but a gallon a day sure isn't. I think what you are talking about is that everything-the effects of compounds, even like folic acid-are going to be dependent upon the dose that you are exposed to per day, and potentially also even genetic mutations in ability to, in effect, metabolize these compounds. Some people might be more sensitive than others to the beneficial or negative effects of folic acid. I don't think this story is done yet. I think everything is like this. If you get too much of something, it is not necessarily good. I always warn people, when I talk to reporters, and make it very clear, because in this country people tend to think if a little is good, a lot is really great. In this situation, we don't know. We do know, for example, that as you get older your epigenome tends to become less stable so it might be advantageous, for example, to have higher levels of folic acid, etc., that would stabilize your epigenome. But we also know that cancer cells, for example, often the promoter regions of tumor suppressor genes are hypermethylated. So it is possible that having too high of doses could actually cause the formation of tumors. These issues just have not been worked out yet.

JB: So when we look at the number of genes-let's say, approximately, 25,000 genes in the human genome that are coding regions for proteins-how many of these genes are under the regulation of promoters that are controlled, in part, by methylation? Do we have any idea?

RJ: I don't personally have any idea. Any gene that is involved, potentially, in differentiation, the promoter regions will have been methylated very early. That is why, for example, a liver cell and a skin cell are going to have a different repertoire of genes that are functional. You have your housekeeping genes that tend to be functional probably in every cell, but then you are going to have specific genes that are involved in making a liver cell a liver cell, and a skin cell a skin cell. Those genes that are involved in that definitely will be methylated, probably in the promoter regions, to turn them off. That is why you can take a liver cell, for example, out of an animal and put it in a Petri dish and it still looks like a liver cell and works pretty much like a liver cell. It's a memory system, basically.
JB: You have raised a very interesting question, obviously, because if there are those imprints with methyl groups that are very tight and not likely to give up their legacy, then there may-by your suggestion-be some methylated regions that are more, maybe, labile to demethylation that can be put on and taken off. It would seem like both of those things are occurring within cells, some more labile than others.

RJ: Right. And there are going to be other genes-I think of them as more equal than others-and, as I said, the group of genes that I am particularly interested in and our lab is working on very, very diligently right now are the genes that are called imprinted. The reason why this is also very, very important--and a point that we really haven't made yet--is that the epigenome varies greatly between species. When we first started out the conversation we were talking about this viable yellow Agouti mouse, where you can switch from yellow to brown and even have calico-cat-looking mice that are in between. There are other strains from a C57 background (which is a strain of mice) that don't have this transposable element. Upstream of the Agouti gene you are never going get these phenotypic changes in response, to, for example, methyl donor supplementation. So the point I am making here is that it is going to be very difficult, or more difficult, to extrapolate between species concerning the effects of environmental conditions on the epigenome concerning the role of how they affect phenotype between, let's say, mouse and humans. Because the epigenome is different. As I always say, if we had the same epigenome as a mouse we would have a long tail and a snout and we don't, right?

JB: That's right.

RJ: They are very different. So the targets, the epigenetically labile targets, will potentially vary-not always-greatly between species, which makes it very, very important that we determine what are the epigenetic, or labile genes, within humans.

JB: That's a really fascinating idea. So, if we went to Indian corn as a analogy here, which has a lot of transposons, would you expect, then, the coloration from ear to ear of Indian corn to be highly variable related to the environment of that germinating ear of corn?

RJ: Compared to...

JB: To regular cultivars of corn, because you are linking the transposons to these more imprintable genome characteristics.

RJ: Correct. Yes. The phenotype would be different because the transposable elements don't even have to be in the same locations in different strains of corn, I would think.

JB: That's very, very interesting. Yes. Absolutely.

RJ: I mean, that transposable element is upstream of the Agouti gene in the viable yellow mouse, but it is not upstream of the Agouti gene in a C57 mouse. And with imprinting, we know this now and there is good experimental work now with predicting the genes that have high probability of being imprinting in mouse and human, for example. We predict that about 600 or so in mouse and about 156 in humans, but what is even more important is the overlap is only 30{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36}, so the repertoires are very different.

How is Dominance Determined?
JB: If I could just take one step from this discussion to the question which has been on the mind of every kind of amateur geneticist: if we are diploid beings, getting one set of characteristics from our biological mother and another strand from our biological father, then who wins the battle to get expressed? We have always kind of thought of this kind of simplistically as "the stronger wins," so whatever parent brought the characteristic that was dominant to the other wins, but this imprinting characteristics suggests that maybe there are other variables going on as to which strand of our DNA wins.

RJ: Yes, I mean, let's say, if you even went to the Agouti mouse kind of thing with transposable elements...in our system, we only have that transposable element and it is the copy that is inherited through the father; the mother's does not have it. But if you had it on both copies, both chromosomes, you could have both copies turned off, one copy turned off, or none of them turned off. There is a lot of variability, here, in expression, just with that type of system. Now with imprinting it is even crazier because the turning off of a copy is dependent completely upon which parent you inherit it from. IGF2 is expressed only from the father's copy, so always the mother's copy of IGF2 is turned off.

JB: That's very interesting.

RJ: So it is monoalleic expression in a parent-of-origin-dependent manner. So imprinted genes actually have a sex. Don't you love it?

JB: Absolutely. I would just like to ask you the obvious. What does this do to generations of geneticists as it relates to what we answered on test questions to get good grades? It sounds like there is a lot of stuff we have to correct here in terms of the way we look at genetics.

RJ: Most genetics that we learned is correct, but there are some interesting things going on that don't obey the standard Mendelian inheritance. Imprinting is one of them because, think about this, how do you-generation after generation-only have the mother's copy turned off for IGF2 and another gene like (at least, in mice) IFG2 receptor, which is a degradation pathway for IGF2, only the mother's copy is always expressed and the father's copy is turned off. The inheritance of that is very, very different. In effect, what happens is the expression of the gene in this generation depends upon the environment that the gene found itself in the previous generation, either in a male or in a female. It is sort of Lamarckian, from that standpoint. The environment is very, very important in determining whether the gene is going to be expressed-totally dependent, in this case.

JB: In your eloquent presentation that you provided in Ghost in Your Genes for the Nova program, at the close of that program-and, in fact, it is in one of your articles, as well-you have an advocacy, which is kind of maybe the consumer takeaway from all of the extraordinary, exciting scientific discovery work. And that advocacy is a little bit about how we see ourselves in the sea of our environment, both from emotion, chemical, air, water, food-all these various factors. Could you share that view with us?

Different Definitions of "Environment"
RJ: I'm not sure exactly what you are referring to. Are you saying about the time that I said everybody has a different definition of environment?

JB: Yes, and that we have a responsibility, which I thought was a very interesting context.

RJ: It's true. I mean, we all have (particularly at universities where groups sometimes don't seem to get along very much...)...you know, if you go to a nutritionist, their definition of environment is basically, you know, the food pyramid. If you go to a toxicologist, it is a super fund site. If you go to...I can't remember what other one I used, even...

JB: The psychologist, with stress?

RJ: That's right, because if you go to a psychologist it is the nurturing environment, and we now have examples, from all of these groups, of people who are doing these types of studies on epigenetics, that these environments are all impacting on the genome and the expression of genes through programming of the epigenome. If you think about the genome as being like the basis of the DNA, as I said, and you have a mutation in that gene, it is very deterministic in that when you have a mutation it is very hard to change that mutation back to normal. The problem is that people-all of us-I think we don't like thinking about having things that determine what is going to happen to us. But with mutations, it tends to be very deterministic. With the epigenome, however, you can vary the programming somewhat by the environment that you create or that is created around you. The problem and the downside of an epigenome of basically someone having free will is there is also responsibility, and I don't know if people

particularly like that part of it. They like the fact that they are not determined, but I am not sure they like the part that there is some responsibility to make sure that your environment is...you know, that you don't do things that potentially harm you or potentially generations of children after you.

JB: So when we look at the body of data that has been collected to date related to methylation and we talk about factors that are related to the imprinting by methyl groups, and then factors that take methyl groups off, is there any sense as to whether it is easier at some of these more labile loci to put the methyls on or take them off or we are not sure yet about the kinetics of this process?

RJ: We are not totally sure. The putting on of methyl groups is reasonably well established through the DNA methyl transferases with the de novo methyl transferases and the ones that maintain it once the marks have been placed, but the removal of methyl groups and how that is done is not totally clear yet. There is some controversy about how this is done. But it is clear that these methyl groups have to be removed because with Moshe Szyf and Michael Meaney's work with licking rats, those areas originally methylated and the nurturing (the licking) of the mother results in the removal of methyl groups and that is a covalent bond that has to be broken and those methyl groups are gone and they are removed in specific areas that allow transcription factors, in this case, that bind upstream of the glucocorticoid receptor. And you have a completely different behavior now.

JB: As I recall, that work at McGill showed the nurturing led to an imprinting so that the offspring, whether they were born to high-stress mothers or not, when they were with mothers that nurtured them by licking and grooming, they ended up being lower stress animals as they grew up.

RJ: That's correct. And if you didn't know that the nurturing was doing this you would just assume that this was something that was being passed through the gametes (the egg or the sperm), right, inherited? But in this case it is not inherited like that, it is the nurturing behavior that is setting these marks, and then they are passed on to the next generation, depending upon what the mother was like. It is pretty amazing stuff.

JB: I would say it is a fundamental shift in our paradigm that has such dramatic, below-the-water line implications for social design...I mean, for everything--our environment, nutrition, how we talk to one another, how we see ourselves. It seems like these things that we have maybe thought were inconsequential and they could just come and pass in the night could have lasting effects, so it is this whole concept of the selfish gene and what we are imprinting on the selfish gene...seems like it may make less selfish.

RJ: It is modifying its selfishness, to a degree. It is going to have-I hate to say it-legal ramifications, too, I think.

JB: I would imagine so. As a scientist, I know it's not fair to put you on the spot, to look forward and kind of forecast out where this might go, but, you know we are talking to clinicians, principally, people who are seeing patients everyday and helping them make intelligent decisions about their lives. What would you say, at this point, is a responsible takeaway from all of this extraordinary work?

RJ: You mean how clinicians will be treating diseases?

JB: And how clinicians might language this to their patients in terms of people who have chronic lifestyle-related diseases.

RJ: You mean changing their behavior, essentially?

JB: Right, exactly.

RJ: What is going to have to happen, I think, in order for people to, in a way, to sort of believe that this actually can occur, is it has to be situations where, for example, you have real problems that you can identify, that can be potentially reversed through psychotherapy, nurturing, that type of thing, and then have a readout of the epigenome where you show that you have actually changed the programming of genes, let's say stress receptors or something like this. Why are the Agouti mice so important? They are the poster children for the importance of epigenetics because you can see it. And I think that is going to have to happen before people are going to be willing to change their behaviors-that you can show that this is what is going on, not only do you feel better, but you have made a permanent change in your programming. Not everybody is going to be impressed with this, but I think it is going to be impressive to a lot of people. I don't think that is that far away, frankly. These types of studies are going to be done. And the other thing that is already being done is the use of what they call epigenetic therapy for the treatment of cancer. It is possible that some of those same things (or comparable things) will be used in the future, for example, to treat neurological disorders, which at this point is not really thought of much, but I think in the future this could be the case. We might be able to reprogram with the use of compounds that alter the epigenome.

Future Research into the Epigenome

JB: Unbelievably exciting. So in the call-to-action at the end of the Nova program there is the quest for deciphering the epigenome, but of course, as we know, this is complexity upon complexity, each cell being different, with regard to its epigenetic pattern. Where will we be, do you think, in the years to come with regard to taking bites of this apple of understanding the epigenome?

RJ: Well, I mean, as you said, the epigenome is going to vary in time, and it is going to vary between different cell types because that is why there are different cell types, and it is obviously going to vary between species greatly, too, as we talked about before. So it is a massive undertaking, but I think we have got to start, basically, defining what those epigenomes are, and that is being done right now because there is a roadmap initiative from NIH to, in effect, do that. That is literally just starting right now. I think the first grant applications were submitted maybe about six months ago and I would imagine a half a year or so from now you will have the first laboratories that are going to be funded to, in effect, map out, beginning maybe with stem cells and going up through differentiation the various epigenomes. And those will be used as templates that other types of information can then be layered on top. In the next-I'm only guessing-few years, five years, whatever, there is going to be a lot more of this information available, and at that time, then, we should be able to start determining what is going one when a person has got, let's say, schizophrenia or autism. Are there any of these regions that we now have defined that are specifically altered in the formation of those neurological disorders? I think that is going to be of great importance because you are not going to be treating it from an epigenetic standpoint until you know those diseases and disorders, for example, are, in part, caused by deprogramming of the epigenome. That is going to happen after we learn what the baseline is and we add additional information to it. That is what I see happening.

JB: Well that's an incredibly exciting frontier. I'd like to ask you one last quick question in the couple of minutes we have left. Probably this is a different kind of question than you have had with other interviews. This discovery you have made, in your group, clearly is one of those paradigm-shifting discoveries and it opens up the field in ways that maybe we didn't recognize would happen until the discovery. How is it changing your life? What is the nature of how you see your work and your presence and your advocacy changing as a consequence of this extraordinary discovery?

RJ: Well one thing it does is it makes it more complicated-which is nice-because you are asked to talk to a lot of different groups. I think a lot of the people who are working in the beginnings of this field of epigentics are basically out talking to people and telling them how important the whole field is-so you are busier, from that standpoint. And I have to admit-it sounds terrible-but I had foot surgery done in January, and as a consequence I really couldn't travel, but just even having three or four months of not traveling has been very, very nice. So it has become more complicated from the standpoint of traveling and talking.

I got into the field of epigenetics back in the early 90s because we identifiee IGF2 receptor as being a tumor suppressor gene. And right at that same time, Denise Barlow identified that exact gene as being imprinted. I had no clue what this was about. And when I read about it and realized what was going on, that one copy was always (in this case, one from the father) turned off and it was done epigenetically, I said to the people in the lab, and this was in the early 90s, "Within three years our whole laboratory will be in the field of epigenetics and genomic imprinting." And that is what we have done with the ultimate thought of being able to define those genes in humans that are imprinted and potentially epigentically labile because if you only have one copy working, a single mutation, or a single epigenetic mutation, can actually totally alter the function of the gene. And from that standpoint, I really haven't veered from that goal, and we are very, very close now, I think, to pulling that off and determining that subset of genes in humans that are imprinted, and as a consequence, are potentially targets for environmental nurturing, whatever-deregulation or regulation-by the environment. And as I said, when you can't extrapolate readily from mice, for example, to humans, I think much of our research along these lines is going to have to migrate into humans. As I keep saying over and over and over, a mouse is not a human. And that is particularly true when you are talking about the epigenome.
JB: Well I want to personally thank you on behalf of everyone that has had the pleasure of listening to this interview and all of your work, which is shift-shaping work. Thank you for your diligence. Thanks for you eloquence. And also thanks for your extraordinary commitment to the publications which keep coming out to help all of us who are novitiates in this field to be better educated. We really appreciate the work you are doing and what a marvelous contribution you are making.

RJ: Well I very much appreciate you for inviting me to discuss our work because it is through very scholarly things like you produce that people understand better why this field is so important to human health and disease.

JB: Thank you, Dr. Jirtle, very, very much

Were you as taken by Dr. Jirtle's comments as I? I think there are certain things that happen in your life that are frame-shifting, jaw-dropping experiences. Listening to him was certainly one of those for me. We are witnessing the rewriting of rules through the work of Eric Schadt at Rosetta Merck, and Randy Jirtle, and someone you are going to have the pleasure of hearing next month on FMU. I'll give you a tidbit: Dr. Edward Calabrese will be speaking about hormesis. These people are rewriting the tablets from which

students will learn the new biology that will become the new medicine that will become the new healthcare system that will be focused on function. Isn't that an interesting word? Function. Not just pathology, but function. How does one maintain high-level function throughout the course of living? That is really the challenge to the new medicine: to deliver clinical outcomes that will improve patient function beyond that of just suppressing symptoms. We've milked a tremendous concept for over 60 years in pharmacology, which is a use of toxic molecules to block certain functions to induce the suppression of certain symptoms, and now we are into a new era, leveraging the new biology that will produce the new medicine that is related to improving function and transforming patient outcome. Pretty exciting era. Stay tuned for next month.

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