September 2010 Issue | Randy Jirtle, PhD Jirtle Laboratory Duke University

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Welcome to *Functional Medicine Update* for September 2010. We have been traveling together-those of you who are *Functional Medicine Update* listeners or readers-on a very interesting journey over the past year and a half. And that journey is looking at what previously would have been considered almost antithetical to good science, that is, that our characteristics are totally controlled by our parentage through the traditional Mendelian genetic mechanisms (this dominant and recessive pattern of ancestry that we are all so familiar with). Laid on top of that now is this "new old" concept that there is this imprinting of genes that we call epigenes, resulting in epigenetic changes that then regulate the way that our book of life is expressed as a consequence of exposure in the environment, starting at the moment of conception (and maybe even pre-conceptionally) and continuing all the way through the fetal development period and then into life.

These particular marks that we call epigenetic marks-the methyl groups on the CpG islands--regulate the way the genes are expressed (the so-called "silencing" of genes), or the acetylation of our histone proteins. The book of life is opened at certain chapters, which are read as an expressed pattern.

I'm laying out a fairly broad platform, philosophically, as we get into this issue of *Functional Medicine Update*. What I really want to talk about, from this platform, is how nutrition, lifestyle, and environment can influence developmental epigenomics. I want to talk about how that ultimately may give rise to increasing relative risk to certain diseases, and how those diseases may cluster much more rapidly in populations than we would have predicted on the basis of not having to wait for selective natural selection occurring over long periods of time. Could disease prevalence change very rapidly in a population over time as a consequence of epigenetic changes? I am talking about things such as autism, or attention hyperactivity disorders, or atopy that leads to asthma, or other conditions where we have seen very dramatically significant changes in prevalence that we can't really account for on the basis of the genes changing.

Epigenomics Applied to Metabolic Disorders

With that platform in mind, let's apply this concept to metabolic disorders. Metabolic disorders are probably the most rapidly rising family of age-related disorders that associate themselves with chronic disease and burdening of the healthcare system. These are disorders that used to be found only in older-age individuals and are now starting to be seen in younger-age people. The classic example is what we used to call adult onset diabetes. It was called that because only older people seemed to get it. As the

condition started to be seen more frequently in adolescence, the name changed from adult onset diabetes to type 2 diabetes. It is now a condition with rapidly increasing prevalence in younger people. Why? Have genes changed to the extent that we have undergone mutational injuries? The answer is probably "No." But maybe has there been an epigenetic modulation of the way genes are expressed? That answer is possibly, "Yes." If that is the case, can we do anything to correct the epigenetic marks, and silence the genes that we don't want expressed, and open up for expression those genes that lead to proper insulin signaling, and inflammatory signaling/glucoregulation?

Research Indicates Chronic Disease Can Start *in utero*

Those are very interesting broad questions of health in the public. What about genomic-related issues in the individual and how nutrition he or she is exposed to actually starts in utero and may influence the rest of the course of his or her life? What we are starting to see suggested from some research is that a lot of chronic age-related diseases may actually start epigenetically in utero. With this in mind, we might consider a condition like type 2 diabetes to be a conditionally essential nutritional epigenomic disease that has a long latency period. The latency period is decades, not months like you would have in some infection diseases, or weeks or even days with some very virulent types of infection. But the onset of the disease occurs over decades; slowly but progressively the person becomes type 2 diabetic and may develop cardiovascular disease at a later stage.

Not only are metabolic disorders among the fastest growing health problems worldwide, they also have a tendency for manifestation at earlier ages. There seems to be a higher rate of these metabolic disturbance-related issues in women than in men. By 2020 the number of patients with diabetes is expected to increase to 350 million people worldwide. Obesity now affects between 30 and 80 percent of adults according to a recent World Health Organization European Region study. Also according to this report, up to one-third of children were already classed as early-stage obese. Unfortunately, more than sixty percent of children who are overweight before puberty will be overweight in early adulthood. Women with diabetes and obese women are sub-fertile, and we know the frequency of polycystic ovary syndrome and anovulation and fertility issues (reproductive biology) is adversely affected. And women with diabetes have a higher risk for spontaneous abortions and congenital malformations in their offspring. Looking at data, increased BMIis associated with adverse effects on lactation and mammary tumorigenesis. Set against this context of a worldwide epidemic is the increasing rate of overweight individuals who have imbalanced nutrition in women of childbearing age. According to the WHO report, in a recent study 25{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} of women in France and 50{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} of women in the United States were found to be in a nutritional status that could predispose a fetus to subsequent metabolic and epigenetic misprogramming, and thus lead to common adult disorders that includes things like metabolic syndrome, type 2 diabetes, and cardiovascular disease. This is almost like an epigenetic nutritional epidemic that we're talking about.[1]

The developmental origins of adult health and diseasepotentially tying back to fetal origins was actually first hypothesized in the 1990s. According to the same article featuring the WHO data, there is compelling evidence that specific ontogenetic changes, such as prenatal development and early childhood could be an obesogenic environment. This environment could then program children to go on to express obesity and obesity-related metabolic disturbance disorders like type 2 diabetes in their phenotype. It's not that these children have the genes, per se, for obesity, or the genes for diabetes. Rather it is that the obesogenic environment in utero created a milieu that then altered genomic imprinting to result in the

expression patterns, postnatally and over time, of a sequence of events ultimately associated with obesity, heart disease, and diabetes.

These trends are continuing and that is bad news. The good news is that if this model that I have just proposed is correct, this is a reversible trend. By doing the right thing, you can alter the epigenetic marks that are put on the genome during fetal development at these labile regions that interrelate with disturbances that we call metabolic diseases, and prevent these diseases from being manifest.

Some research has indicated that epigenetic effects induced during the perinatal period produce persistent development adaptations in structure physiology and metabolism. Animal work in this field has been published over the last decade. How does an adult organ's genome retain the memory of the intrauterine (or early life) exposure long after the exposures have been taken away? I have discussed bisphenol A as a potential obesogen that may have effects on imprinting the genome epigenetically in the fetus in such a way that it alters, in the fully developed organism, expression patterns of various physiological or metabolic functions that are associated with disease.

It could be the chemical environment. It could be the nutritional environment. For instance, women, if they are folic acid deficient/methyl deficient upon conception and during pregnancy, can undermethylate and that can lead to increasing risks to the silencing of various genes. When expressed, these genes can lead to increased malformations, like spina bifida cystica, or anencephaly, or altered kinds of conditions related to the expression of oncogenic potential that could lead to increasing risk to cancer. These methylation patterns play roles in regulating, epigenetically, the expression of function in the adult after birth. And those methyl groups are, to some extent, dependent upon the availability of s-adenosylmethionine, which, in turn, is dependent upon the adequacy of the methylating nutrients (folic acid, B-12, B-6, betaine) in the periconceptual woman's diet. All of these things are potentially starting to cause us to change our view of changing disease patterns occurring in our population at a fairly rapid rate, not just in the United States but globally.[2,3]

The important thing, as I mentioned, is that these epigenetic marks are flexible; it is possible both to put them on and to take them off. By altering environmental, nutritional, social, cultural, and hormonal factors, and by changing drugs and toxins, you can alter the epigenetic landscape during this spaciotemporal window in which tissues are being formed and differentiated, and ultimately leading into a sex-specific phenotype. This is why females might have possibly higher sensitivities to some of these epigenomic regulators than males; it is a consequence of the differentiation of their developing endocrine system. Alternatively, other alterations of epigenetic marks can lead to irreversible changes in lineage specification in the deviation of a cell type determination, which could lead to oncogenic potential and increasing risk to cancer. It could also result in, as I said, long-term latent periods that don't express until late teens, 20s, or 30s as a disease that has come from that dysfunctional phenotypic expression pattern.

I think these are very remarkable concepts that are more than theories. There are adequate information and data to support doing evaluation of the epigenome and looking at aspects of methylation or promoter regions of genes. Researchers are looking at histone acetylation in genomes and actually correlating, in animals and humans, altered states of methylation with health outcome.[4,5]

I believe that those individuals who are holding on to believing the fixed mechanism of disease causation is locked inextricably in the genes are making specious presumptions and are way too rigid in their views.

As I said, as we learn about systems in life more and more, things seem to be in-between two polar opposites. Rather than being black or white, we're most often in some degree of gray, and that gray can vary in shade depending upon the environment.

How do we appropriately regulate the epigenome? Many of the epigenetic marks that are laid down during fetal development actually appear to be transmissible into the next generation through reproduction. So regulating the epigenome may not just be about influencing the initial generation. There may be a transmissibility factor that moves into the next generation in the absence of something changing those marks.

I am reminded of Lucille Hurley's work at the University of California at Davis that was done some 30 years ago. Epigentics might have played a role in her work on zinc. In her study, which was an animal model, she deprived the mother of zinc during the early stage of pregnancy (not to be frankly zinc-deficient so that the mother would be in serious compromised/imminent death, but marginally deprived).. The offspring of that mother that was marginally deprived of zinc had immune dysregulation; they were more allergic and they were more atopic. If that was continued on for another generation, it got progressively more severe.

This sounds a lot like the Pottenger cat studies, doesn't it? In the 1930s in California, Dr. Pottenger was rearing cats on cooked meat and milk and showing that they developed allergic phenomena. He was able to produce, actually, the first arthritic cat by subsequently feeding three generations of this-what he called-"devitalized" food to the animals. Lucille Hurley's work seems reminiscent of Pottenger. I In her controlled experiments it took three generations of repletion of zinc to bring those animals back to the same immune competence as the initial mother.[6]

How long does it take to correct epigenetic marks that are laid down? I don't think we have a simple answer because it depends on the mark, its location, in what tissue it is found, and in what period of development it was altered. There are myriad complicated variables that might change, to some degree, the way we answer that question. I think what we can say is that certain marks that are laid down can be taken up, and other marks can be laid down over time. That would be things like altering the environment, wouldn't it be? This concept is about asking different questions to get different answers, not just rearranging the deck chairs on the proverbial metabolic Titanic. It is about making the appropriate changes by changing the exposure to certain xenobiotic chemicals (for example, moving away from some of the xenoestrogens like the dioxins and some of the bisphenol-A-like compounds that influence hormonal development epigenetically), and changing dietary exposure to obesogens, the environmental epigenomic nutritional programming agents. It is also about improving the presence of adequate nutrients that are necessary for control of proper epigenetic programming, like the methylating nutrients and also things that control histone deacetylases and acetylation, such as butyrate in the gut..

Clinical Utility: Asking Different Questions Could Lead to Different Answers

What I'm really saying is that asking different questions could lead to different answers, which could then alterepigenetic programming. From a functional medicine perspective, I think this is quite an interesting topic that has clinical utility. We talk about function being the result of the interactions between genes and the environment. The environment could be things like exercise, work, stress, toxin exposure, lack of life fulfillment or love, lack of attribution, poor quality diet, or it could be exposure to cigarette smoke or lead. All these various factors are modifiers (maybe we should call them biological response modifiers)

that alter the way that gene pluripotentiality is converted into the phenotype (either the healthy phenotype or the not-as-healthy phenotype). But now we have a weigh station, or a weigh point, along the road. We're saying it is not just modulating the metabolism, in and of itself. It is actually modifying the way that the book of life is read into metabolism. We have to look at the genomic, proteomic, and metabolomic factors all working together to give rise to the phenomics-the outcome of that individual-that is expressed over time as they grow older, and go through their developmental cycles. We can have long-latency chronic diseases where the root origin started at much younger age and we just didn't know it.

This is a concept Robert Heaney talks about. He is the professor of endocrinology from Creighton University Medical School, who recently received the McCollum Award from the American Society of Nutrition, the United States' most prestigious nutrition investigator award. Dr. Heaney talks about long-latency nutritional inadequacy diseases, like osteoporosis. You don't get osteoporosis overnight if you don't get vitamin D and calcium, but over time it expresses itself. Similarly, you don't get diabetes immediately as a consequence of altered insulin dysregulation; you get type 2 diabetes over a period of time. Similarly with coronary heart disease. Maybe even similarly with certain forms of cancer that may take 20 years from the root origin of change at the cellular level to be finally expressed as a diagnosable cancer.

All of these things, to me, are very important. The vitamin D story is a good example of this concept. Vitamin D, as we have talked about in previous issues of *Functional Medicine Update*, influences nuclear orphan receptor effects and the expression of many genes. People have been in debate about exactly how many genes are turned on as a consequence of 1,25-dihydroxyvitamin D3 activity, but it is certainly tens of genes that are modified in their expression when 1,25-dihydroxy D3 hybridizes with things like triiodothyronine, or the retinoic acid receptor, or even retinoic acid itself. It then activates specific genes, that then produce mRNA (messenger RNA), that then are converted into certain levels of active proteins, that then undergo post-translational modification to form active proteins, and then alter metabolic function and cellular phenotype. That particular sequence of events that I've just described is ultimately tied back to an environmental factor called the sun.

Photobiology. Lack of exposure to the appropriate wavelengths of light in the skin (and depending upon the skin color) converts dehydrocholesterol ultimately into 25-hydroxyvitamin D3, which is produced by hydroxylation in the liver, and then that gets further hydroxylated to 1,25-dihydroxy. It can either happen in the vascular endothelium, or it can happen in the kidneys and brain, and then ultimately has this hormonal regulatory effect on gene expression.

We might consider, then, that the environment-in this case, exposure to appropriate wavelengths of light so that the skin can make this material (this vitamin D material)-coupled together with the fact that that material ultimately regulates both gene expression and has effects on epigenomics as well, may set in motion a new set point in physiology that is passed on (a transmissible factor) to the next generation. You might have one generation that has a temporal vitamin D insufficiency that has increased risk to, say, certain neurological disorders or autoimmune disorders (for instance, multiple sclerosis, which has been connected with vitamin D inadequacy).[7] But that effect, if it occurred in pregnancy, could then influence the epigenome of that offspring, which then maybe sets in motion a different regulatory effect upon the offspring.

It is my belief that these conditions that we often see that seem mysterious, where disorders pop up with greater frequency, are not solely a consequence of better diagnosis. I've often heard people talk about the increasing frequency of autism being because we are more attuned to it and we have better diagnosis, but I think it is also a consequence of these epigenetic changes that are occurring that can regulate over time how genes are expressed. This field is so complicated because we are talking about low levels of environmental chemicals that might have influence. We are really talking about levels that are at the threshold of our ability to even analyze them in materials accurately. How do these levels interrelate with levels of other things? Is there an orchestration effect on epigenomics, so it is not just one chemical at a time, but it is multiples at a time? In systems thinking, it is very difficult to unequivocally answer the question of one agent against one outcome. That in itself is challenging, but when you add two agents against an outcome, or three agents, the relative complexity of proving a hypothesis goes up exponentially. It becomes virtually, in atomic theory, impossible to solve unequivocally the three-atom story as to exactly understand the energy of interaction among three atoms together through fundamental theoretical chemistry background. I think we have the same problem when we start looking at chemicals in the environment at low level coupled with altered photoreactivity because of the use of high SPFs in sun formulas, coupled with increasing exposure to heavy metals, coupled with altered stress patterns. I could go on and on with this story.

How do you tease out all those variables but still, as you tease them out, keep the integrity of the system because it is an interacting system in your study so that you actually have something that is realistic, when you've actually concluded your study, about what's going on in life? These are the complicating factors of unequivocal proof of concept, but I think that even in the absence of a complete understanding of how to prove that the system of these interacting variables has a different effect than each individual variable we're able to start understanding that the impact of a changing environment is much more profound, not only in the immediacy of function of that organism, but on the subsequent generations than we previously ever understood.

We're going to have the privilege, in this month's clinician/researcher-of-the-month interview, of going back some two years after we had an "aha" on *Functional Medicine Update* by interviewing Dr. Randy Jirtle, who, with Bob Waterland, was credited-and justifiably so-with making the principal first discovery in the field of nutritional epigenomics. Dr. Jirtle's research group did folate and B12 high-level supplementation experiments in Agouti mice (white fat mice). If they did the supplementation early in pregnancy of the mother, the offspring of those mice, for the first time, didn't have white fur (they had a mottled pseudo-Agouti fur color). And the other interesting thing is the offsping-although exposed to the same ad lib animal chow of their parents-didn't get fat, they didn't get diabetes, they didn't get cancer like their parents did frequently, and they lived longer, without changing their genes, just changing the epigenetic marks due to methylation changes.

That observation, and the photographs of those animals, and the reproduction of that work by many labs around the world has virtually revolutionized our thinking. All the things that I've talked about came out of this "aha-ism" from Dr. Jirtle's group. Dr. Jirtle was one of the principal people described and interviewed in a recent *Time* magazine article on epigenetics published in January 2010.[8]

How long does it take to understand and accept the implications of this kind of a paradigm-shifting discovery? That's what we are going to be discussing with Dr. Jirtle. It's wonderful to go back and revisit with him at what should be the richest time of his life, the most fulfilling time, a time where this field,

with Michael Skinner, who we also interviewed, looked at the effects of pesticides on epigenomics, or looking at the work that hormesis plays on epigenetics (very low levels of substance that produced much higher outcome than we would have expected in terms of changing biological function. That was Dr. Edward Calabrese, you recall, that we interviewed on hormesis (the kind of father of hormesis).

How has this all evolved over the last couple of years as the field has virtually exploded in interest? I'm very privileged to have a chance to go back and revisit with Dr. Jirtle about his extraordinary contributions

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INTERVIEW TRANSCRIPT

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Here we are once again at our Researcher/Clinician-of-the-Month section of Functional Medicine Update. Some of you may remember Dr. Randy Jirtle. The chance to speak to him exactly two years ago, in September 2008, concerning his pioneering work on epigenetics, and how it is shaping much of what is happening at the frontier of biomedicine today was, I thought, a "goosebump" experience. He and I were speaking briefly before we got into today's interview, and I was saying that these interviews with people who are shaping the new medicine is like being in school for me. I'm challenged by the best minds, the people who are really thinking out of the box, the people who somehow have the courageous ability to cross lines despite the possibility of people saying, "Well, you've just stepped out of your discipline, and now you are stepping on somebody else's turf and you should know better."

Randy Jirtle is certainly one of those individuals. As I look at his background, I'm quite amazed that he started out with a BS in nuclear engineering. He then moved to his MS in radiation biology and his PhD in radiation biology with a minor in statistics. Now he is a professor in radiation oncology at the Duke University Medical Center and a world-renowned expert in epigenomics/epigenetics. How does this all fit together? I think that's probably where we want to start.

Dr. Jirtle was the principal person featured in a 2010 article in Time magazine that was titled "Why You DNA Is Not Your Destiny." That was the January 6, 2010 issue. I'm so vicariously proud to see Dr. Jirtle, himself and his work, so prominently displayed in that article.

Dr. Jirtle, let's start. Maybe you can help us understand. How does a person who goes down the track of nuclear engineering into radiation biology ultimately get into epigenetics?

RJ: To me it makes sense because, you know, I've lived it. But I know to a lot of people it wouldn't seem to make very much sense. In 2006-it was a big honor-I was given a "Distinguished Achievement" award back in the School of Engineering at the University of Wisconsin. Because of that, actually, some

interesting things happened. For one, my daughter is now a student at the University of Wisconsin because of going back there and seeing the university. If I would have told her, "You might want to consider going there," I have a feeling I probably would have been told, "No." So that's a positive thing.

I said to a friend of mine, "You know, this is very interesting because I never practiced a day in the field of engineering." I was changing already when I was at the end of my career. I did well in engineering, but I knew I didn't want to stay in engineering to do research as a standard type of research. And at that time, there was no such thing as bioengineering. And my friend said, "If you really think about it, what you really did is you actually did do bioengineering before it ever was around." He said, "That more than likely is why the engineering school finally recognized what you have done and maybe why you have done it because now most engineering schools have very big strong bioengineering departments."

That make some sense to me, and how we got into epigenetics is another one of those "Ys" in the road. When you come to them, Yogi Berra said, "Take it." We took that road back in the early 90s and we identified the IGF-2 receptor as being a tumor suppressor gene. I think it was 1991, and Denise Barlow, who was at Vienna at that time, identified that gene as being the first one shown to be genomically imprinted. That imprinting process involves epigenetic silencing, so it was at that time that I decided we would move our whole research program into the field of epigenetics, because it was clear to me that we were essentially talking about programming of a computer, and I have always liked computers, so I finally felt like I had gotten home.

JB: To me that is the definition of courage: following your intuition, shrugging off convention and maybe even recommendations from your colleagues, and staying the course. You know, you push everything up on the board of life and then you make it happen. Thank goodness for all of us that you made that decision. What has followed, as we described briefly in our discussion back in 2008, is quite remarkable.

In this recent article in Birth Defects and Research in Clinical and Molecular Teratology (this was in the June 21st issue) that you co-authored with Bernal, you really, I think, in the introduction of that article, say some things that are not just exciting, they are truly paradigm-shifting.9 So many of the things that we thought were facts and immutable--things that we took tests on when I was back in molecular biology and molecular genetics in the 60s-are now being set aside. I'm going to quote from the article: "Through DNA methylation, histone modifications, and small regulatory RNAs, the epigenome systematically controls gene expression during development, both in utero and throughout life. The epigenome is also a very reactive system. Its labile nature allows it to sense and respond to environmental perturbations to ensure survival during fetal growth. This pliability can lead to aberrant epigenetic modifications that persist into later life and induce numerous disease states." I'll stop there. That collection of words, to me, represents a landscape of change that is truly remarkable. Can you summarize for our listeners what's meant by that? It's just profound to me.

RJ: First of all, when you read it back to me I was thinking that I should have put a period in there somewhere!

JB: It may be the way that I read it!

The Epigenome as a Programming System
RJ: As I said, it's a programming system. We have one genome, half of it comes from the mom and half

of it comes from the dad. I think of that as sort of like being the hardware of a computer. That's the analogy I always use. But we have 250-300 different cell types, so how do you get those different cell types? You have to tell each cell how to work, and that working system, which we collectively call the epigenome, that's the software that tells those cells how to behave and work once that developmental process has occurred.

A liver cell doesn't have to have tissue bumping up against tissue to make it into a liver cell every time it divides; the program has been set during that very early developmental stage. As a consequence, you can even take a liver cell and put it into culture and it still remains a liver cell. So you have a programming system, just like you have in your computer, that allows that to occur.

But once you start using-in effect-software to control cell type and what a cell does, now you've got a system that is intrinsically more labile than the physical hardware of your computer. It doesn't mean, for example, that a hard drive couldn't go out or even a chip couldn't go out, but it is much less likely that that is going to be problematic and cause problems for your ability to use your computer than when you get a glitch, or a bug, or something (a virus, for example) in your software.

So it is a labile system. That labile system has pluses and minuses. It also allows that cell, now and during development, to respond to early environmental changes. You don't have to rewire something hardware-like to change the way it works; the environment can do that to a certain degree.

I'll stop there because often I talk sort of in a run-on way. I want to have you add your comments to that.

JB: I think that the takeaway that I'm starting to better appreciate and understand from your work is that there are many variables that early development can be exposed to (early fetal development can be exposed to) that can modulate or imprint and result in different functional phenotypes that may last a lifetime. We had the opportunity to interview Dr. David Jacobs recently about his collaboration with Dr. Duk Lee. I know you are very familiar with their work around environmental exposures and the influence that they have on later-stage chronic disease. It seems that much of what they are observing, to me, is really overlapped with the epigenomic imprinting that you have discovered. Do you feel that there is kind of a convergence between these two schools of research?

Overlap of Research with Dr. David Jacobs and Dr. Duk-Hee Lee

RJ: Yes. These systems didn't evolve to give us problems. You know, scientists...I guess this is maybe a negative...we think somewhat critically and therefore you think always negative about the bad things that happen. But this system, when you are allowed to, for example, respond to the environment, it enables the developing offspring to potentially set itself up to better function in the world that it perceives it is going to be living in. The problem that we see now, particularly-and this happens with all species because any time you have differentiated cells you've got to have some sort of programming going on-is that there is a perception in many societies when you are in utero that you're going to be in an environment of low amounts of nutrition, etc. But we're finding now that we're in the land of plenty, so what is becoming epidemic throughout the whole world, not just in the United States or Western culture, are the problems of obesity, diabetes, and increased incidences of cancer. It is a mismatch that appears between what was perceived to be the environment that we are going to live in and the environment we find ourselves in. It is probably one of the first times--or maybe the first time--that's ever happened because usually an overabundance of food is not what our species has encountered.

JB: Let's take a specific example. I know you have authored a number of papers that have discussed this, and this subject is also related to things that Jacobs and his group with Duk Lee have looked at, and that's environmental exposures to things like bisphenol-A, which is an epigenic-marking substance, and it would be an epigenotoxic effect with BPA.10 But you've also found, at least in your animal work, that you can ameliorate some of that effect of BPA on the epigenome by supplementation of the pregnant animal's diet with methylating nutrients or with genistein from soy. Could you tell us a little bit about that?

RJ: To me, this is the hopeful part of this whole story. Hippocrates said two millennia ago, "food is medicine," and that's what we, in effect, have shown, at least with these doses of exposure to bisphenol-A and the doses of supplements that we used in this animal model. I say all of that because epigenomes vary greatly between species, so the ability to potentially extrapolate between species is going to be a little bit more difficult, I think, than extrapolating mutational effects between species. I really think that this whole field of epigenetics is very positive because it demonstrates that there is the possibility of preventing many of these problems, and that's an extremely hopeful situation because if you have a mutation and you inherited it, you can't change that mutated base back to normal, whereas we potentially can alter epigenetic programming, or even potentially, when the programming is there, reverse it. This is prevention, and I think this is what we are going to start seeing people think about and do much more than they have done in the past.

JB: Let's take an example in an area that I know you're a world expert on, and that's the whole oncology area. Let's look at Mary-Claire King and BRCA1 and 2. I've had the privilege of speaking with her as well. I think she's a quite remarkable person on a lot of levels, both as a molecular geneticist and also as kind of a scientific philosopher. She made the observation-I think she even stated this in one of her papers in Science magazine a few years ago-that in the late 60s/early 70s, women who had the BRCA1/2 homozygous recessive "bad luck of the draw" would go into the phenotype of having the probability of breast cancer somewhere around 50-plus percent.11

RJ: Right.

Can Epigenetics Be Altering Disease Penetrance?
JB: But with that same genetic characteristic now in the 2000s (the early 21st century), the expression and penetrance into the phenotype is like 90{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36}. So the genes have remained constant, but their expression patterns have changed dramatically. I know you've looked at some of the epigenetics. Do you think there is a factor of epigenetic silencing that controls the expression of things like BRCA1 and 2, which we consider almost immutable deterministic cancer genes?

RJ: Yes. I get this often, but the way you stated that is really beautiful because you get goosebumps truly thinking about this. We always think about the percent of the people that get (or have) the problem from inheriting these mutations, but we often don't think of the other side. In other words, there is a certain percentage of people that, for some reason, don't have these problems even though they have the mutations. People have thought (scientists have thought) that there have to be "modifying" factors that in effect, as you say, reduce the penetrance of the mutation. In other words, you have the mutation but you don't see the phenotype. I think many people thought that these modifiers (again, why they worked or didn't work) was because of mutations. In other words, there is something else that is modifying the effect

of having the mutation in the BRCA1 and BRCA2 genes, and that something else (let's say a gene product or something like this) that, again, is mutated or not mutated and that's why you have this variation.

But the other possibility is whatever these modifying factors are, they could literally be controlled epigenetically. So now you have the potential of altering penetrance not by changing at all the BRCA1 and BRCA2 mutations, but changing the expression of the modifying factors, and you could do this very rapidly, and with high probability through a population, through epigenetic phenomena. Whereas you would never be able to see that with that great degree of effectiveness by causing mutations in those modifying factors. You just wouldn't see it because you'd have to inherit, again, and usually these mutations are recessive, so you would have to have two of them come together to have an effect.

You can see what I'm saying. It doesn't fit very well with Mendelian inheritance. With epigenetic phenomena, particularly if you can even pass that forward from generation to generation through the gametes, you could very rapidly cause a very marked effect on the ability of this BRCA1 and BRCA2 mutation as causing a problem.

JB: Now you've really opened Pandora's box with me, so bear with me. I'd like to follow-on several of the thoughts you just left us with. You very gently and graciously talked about what I guess we would term "transgenerational epigenetic transmission," which in the face of strong Mendelian thought sounds a little like what I would call an adaptation. Or it sounds like things that we were told in genetics just didn't happen. Is there evidence supporting this transgenerational epigenetic transmission?

Evidence for Transgenerational Epigenetic Transmission
RJ: Definitely in plants and in insects. It is very clear that this is the case where things have been through for tens to hundreds of generations: variegations in plants, structure of the flowers, eye colors, and different types of things that you see in fruit flies, for example. These are epigenetic and they are passed forward from generation to generation. There is no doubt about that in these species. The problem is it is not quite as clear whether you can have these types of inheritances occurring in mammals. I think, for example, with the Agouti mouse model that we used, where you have this coat color change, there is evidence that even in this model that this information is passed forward from generation to generation. But it is not as clear, I don't think right now, at least not at this point, that you have this transgenerational inheritance in mammals as you see in other species.

JB: Looking back just historically for a moment, I think Jean-Baptiste Lamarck and Darwin were contemporaries, or at least close in age or close during the period. Why did Darwin so predominate in his concept and Lamarck look like an artifact?

RJ: I'm not really sure. I'm not an expert in evolution, but I don't believe that Darwin totally dismissed Lamarck's theories back when he was writing, but for some reason they just didn't catch on as readily. Maybe it was because of Mendel's work at that time and that people focused more on the mutational aspects of things rather than these programming changes. You've got to remember, 30 or 40 years ago when you didn't know how something worked you'd say, "It must be epigenetic," which basically meant, at that time, that you had no clue what it was so you just threw it into that box. That gave sort of a bad name to the whole field, plus there were political things that occurred, too, that went against Lamarck-type theory that people didn't like. But now that we have much more information about how these

programs are controlled by DNA methylation, histone marks, etc., this field has gone forward very strongly, and I think that has helped a lot; it is not discredited quite as much.

JB: If we look at this imprinting that you are talking about, and let's go specifically to methylation for a second, one might consider silencing of genes, if it is an oncogene, to be possibly a good thing. But then one might say, "But hold it. If you methylate and silence the promoter region of a tumor suppressor gene that might be bad." How does one address that philosophical conundrum?

RJ: Yes. The problem with everything in this whole filed is dosage and timing. In terms of what might be good at a low dose, I use the analogy of a glass of wine or two a day might be good for your cardiovascular system, but a gallon a day probably isn't. And the other thing is that there are particular times in our lives when we are more vulnerable to changes that could be deleterious than other times, and particularly, I think, from the standpoint of tumor suppressor gene inactivation in cancer. This is a disease of the aged, primarily, so as a consequence, having high levels, let's say, of folic acid in our flour has been shown to be very good from the standpoint of reducing neural tube defects. But at the same time, for another population of people that are here which is people that are older, it could potentially increase their chances of getting cancer by epigenetic silencing of the tumor suppressor genes. I don't know how you get out of this problem because not one set of clothing, basically, fits all people.
JB: That raises a question for me. You and I just touched upon this two years ago in our interview, and that was that the fetal genome is very susceptible (open) to epigenetic imprinting, so in the first trimester it is critically important to make sure you prevent bad exposures to the fetus, and get the right nutrition and the right balance. But then we talked about the question, "Are there labile epigenomic marks that later in life can still be manipulated by an altering environment, or is it that once set in the fetus it is locked in and immutable?" I know you have come quite a ways in the last two years in the field in understanding what is and what is not labile as we get into a fully developed offspring. What is the status today?

RJ: From the standpoint of the Agouti mice, the question I get often is, "Can you affect coat color, let's say, after an animal is born?" In other words, if an animal is born yellow, which means that little bit of DNA that is upsteam of the Agouti gene, during early development, wasn't methylated, and as a consequence the Agouti protein is produced all over the body and gives rise to a yellow coat. In those animals, if you continue them on a high-methylating supplement in the diet, could you gradually change that coat color to brown, for example, in this model system?

We have not done those studies, but I have talked to a colleague of mine about it. I don't think this has been reported. He asked, "Have you ever done that?" and I said, "No, because 21 days after they are born those animals are gone because animal care costs are high so we don't keep them for long periods of time." But he said, "We did." And he said, "I've taken pictures of them." And he said, "When you keep them on these high-methylating diets, you can see that the coat colors gradually start becoming browner and browner," suggesting that you can alter these epigenomes even after birth. That hasn't been reported and I have not personally done it, but that is one set of observations that suggests to me that you can change things even later in life.

Whether you can change it with the kinds of things that we can ingest or if you have to use different types of compounds that are much more effective in doing this I'm not sure. But we know we have epigenetic therapy for cancer, so there we are causing changes in the methylation patterns after the tumor has developed. You can release those methyl groups and, in effect, induce tumor cells to commit apoptosis

and die. So I think it is probably true that one could do these types of things also in a normal individual. Maybe it is one of the reasons why we do have increased incidences of cancer. If we're not maintaining our epigenome properly, we might ultimately have problems. All of these issues just have not been addressed yet very well. We're just starting. I think with the tools that we have right now for looking at the epigenome-the sequencing tools, basically-a lot of these questions are going to start becoming resolved, but we don't have these issues resolved at this point.

JB: I think you've really done such a superb job of laying out what the present state of our understanding is and the potential significance of this playing field, of this whole filed. I think one of your colleagues at Duke Medical Center has done quite a bit of research on...I can't remember the name of the drug, you can help me with it, I'm sure...a drug that alters methylation. I think you were just referring to it. It is used in chemotherapy for specific tumor types. Maybe you could tell us a little more about that, because to me that is seemingly an interesting example of adult modulation of the epigenome.12

Epigenetic Cancer Therapies

RJ: They are able to treat certain types of leukemias with what they call epigenetic therapies. Many of the tumor suppressor genes are hypermethylated (or they have many methyl groups in front of the tumor suppressor gene). When you expose them to these compounds, what happens is those methyl groups gradually are released, and as a consequence, now the gene functions and you induce that cell to either be a good citizen and stop growing, or usually what happens is it undergoes what is called programmed cell death and they die. So you are trying to make them good citizens. That is different from most therapies. Chemotherapies that we are using now are cytotoxic and they just literally sort of nuke the cell. The same thing with ionizing radiation; I mean, they just blast the cell. So this is a very different approach. Lower doses of compounds are used (of these therapeutic agents), and for longer periods of time. And you don't see, in these patients, the negative effects that you would often see with the cytotoxic agents.

It's a whole different field. If there were epigenetic changes, for example, that gave rise to autism, or let's say schizophrenia and other neurological disorders, it is potentially possible we could use compounds that would alter the epigenetic states of genes that are very important in giving rise to this and therefore block this problem, and as a consequence, get people through a developmental stage to the point where they would never have that problem at all? I think this the exciting part of the epigenetic therapy and the epigenetic field of scientific research. Again, it is prevention. Even though what we were talking about initially with cancer is therapeutic, I really think the big thing is going to be ultimately the use of compounds, and nutrition might be one of those types of things to prevent these problems from ever occurring.

JB: To me that's obviously really the exciting frontier. Is it appropriate to call those alleles that might be modifiable something like "metastable epialleles"? Or is that not appropriate language to define them?

RJ: That's the terminology that was coined for these Agouti genes that can be methylated or unmethylated. They are referred to as metastable epialleles. There are only-to my knowledge-three genes that we now know of in different strains of mice that are regulated by this type of metastable effect. In humans-to my knowledge, again-we don't know any. That doesn't mean that there aren't some, but it's going to be a little more difficult to find them because every individual also varies in his or her genome, so it is going to be harder to determine whether an effect that you see is because of a genetic change or difference or because of an epigenetic change. So they are harder to find but I believe they are there, and

we now have the tools that I think we should be able to find these things.

One thing that is really interesting that is coming out of a lot of this work now is copy number of variance. In other words, you have areas where there are microdeletions or microamplifications. It's possible these are occurring in areas where one allele or one copy of a gene is silenced (in other words, these imprinted genes). They can be either imprinted genomically, so you have parent-of-origin effects of the expression, or they could be caused by mutations and could be altered in their expression because of genetic sequence that is close to them, but it gives rise to a lot of genes, I think, that are expressed only from one copy. If you end up deleting that one copy, you can see very clearly why you would have an effect on phenotype.

JB: Yes.

RJ: It's really interesting because I feel we're going to find a lot of diseases are mapping into these regions where we have genes in which only one copy primarily functions normally.

JB: Yes, and I think that's a part of genetics that many of us, in school, didn't really consider the implication of. We get something from our father, we get something from our biological mother, they code together. Which of those two sets of characteristics get expressed? How do they get mixed and matched? What do the promoter regions look like? Which are silenced; which are activated? And do they change in changing conditions so that one gets turned on and the other gets turned off in its expression? It's a lot more genetic variability potential into the expression, it would appear, than maybe we first recognized.

RJ: Right, and that information is coming out now because we can do this massive sequencing and look at not only methylation differences between the two alleles, but you can very clearly quantify expression of the two copies if you have some sort of a mutation or polymorphism, as we call it, between the two copies. So you can see whether one copy or the other copy is expressed more prevalently, or if they are basically expressed sort of equally, which is was most people thought (if genes were on, both copies worked).

Hypermethylation versus Hypomethylation

JB: Two last thoughts here. We could go on-at least I could go on-for hours on this. Your work just opens for me so many interesting thoughts and questions. Let's come back to the word that you used earlier, which is "hypermethylation" as contrasted to "hypomethylation." There is this view, I think, that if you don't get enough methylating nutrients that you put at risk regions of the various CpG islands and the promoter regions of genes to be hypomethylated and to not have proper silencing. And then the alternative to that is people might say, "Well, if not enough is not good, what about too much?" So let's say we stimulate too much methylation by giving too high levels, as you were implicating, of folic acid or B12 or B6 or a combination there of, and you get into hypermethylation? It appears to me now, from what I'm reading, that when you have a state of hypomethylation in specific regions of the genome, you have hypermethylations in others. It's not just a one-size-fits-all, too low levels leads to always hypomethylation. So it sounds like altered methylation occurs in the genome as a consequence of factors in the environment that modify the methylation pattern. Is that what's emerging?

RJ: Yes, it seems like it. In particular, that ends up being the case in cancer formation. The first thing that

was defined, back in the 80s probably, that you saw in cancer formation was horrible hypomethylation. But then the whole emphasis went towards the fact that some of these tumor suppressor genes are silenced because of hypermethylation. Now we know it is exactly what you said. In this sea of hypomethylation, you do have certain areas that for some reason become hypermethylated. So you have all of these combinations. Basically what it is is a deregulated epigenome, and it is causing a lot of different changes, of which some of them, then, ultimately are selected for because of the growth advantages to those cells and they ultimately are what we see as a tumor.

JB: What I'm starting to better understand is that maybe these patterns are really reflections--the shadows of the image of altered cellular regulation from different environmental perturbations.

RJ: Right. They are deregulated in general, and then you get variations of regulation. Why do we look at cancer and see these? Because they grow bumps and they kill you (not all of them; at least some of them do). So you have natural selection for certain genes being turned off and certain ones being turned on, sometimes through mutations, but also sometimes because of alterations in the epigenomes that are controlling the expression of these genes. When you select for something that gives you a net inappropriate growth that's not controlled the way it should be, you end up with cancer, and it is natural selection for these different alterations in gene regulation.

JB: Let me, if I can, close with one last question, which maybe is an overarching question that ties a lot of this together. You've talked, in some of your recent papers, about epigenetic biosensors that then control regulating genes. Often these are in the non-coding regions of genes-very interesting-in the entrons that are the regulatory regions that we used to call junk DNA, which I find kind of interesting just from a historic perspective (be cautious what you call junk).

RJ: Right.

JB: One of the things that struck me as I looked at this, is that this imprinting that occurs in fetal development implies, as you have shown in your work,, that for many diseases that we get in later life, the clock started ticking with our fetal epigenomic imprinting: diabetes, CVD, and cancer might come many years later. So they are diseases of long latency from epigenetic modulation. As you looked at this, do you have a sense as to whether most chronic diseases are a consequence of the epigenetic imprinting at the fetal stage, or is it not yet known how much of this is modulated later in life?

RJ: I don't think we know because we really just don't know very much about it. This is a guess, now. People say, "What's more important, mutations or epigenetic changes in disease susceptibility?" And I say, "You really can't ask that question. You can ask it, but you can't answer it." Because if you use the analogy that I have used about the hardware-the DNA-being comparable to the hardware of your computer and the software being comparable to these epigenetic programs, and if you are typing on the computer, you could ask, "What's more important to you right now, that physical computer or the software program that you are using to type that information into the computer?" They are both important.

But the other question that you just asked is what gets messed up more readily and potentially gives rise to problems? I think that we're going to find that what's causing the vast majority (or at least a goodly amount of these problems), are not going to be just these mutational effects, but frankly will be these

epigenetic changes. They are more labile, and therefore as a consequence it is more likely that you can change in a bad way. So that's my guess.

JB: I'm obviously just an armchair observer of what you are doing as the expert, I've looked at what's going on with Mike Skinner and the work that is going on at McGill with the stress-related factors and the epigenetic imprinting in animals and the relationship to later-stage disease.13,14 Now that these tools are available, this seems to be growing exponentially to give much more weight to this plasticity that is regulated through the epigenome, as contrasted to the hard-wired change of mutation in the traditional kind of Darwinian/Mendelian sense. This is a whole field that is emerging, which I think has a good news component to it because if we, as you said, can identify where these loci of alterations are and look at how you can modify them favorably by regulating the environment, it may lead to reversibility, which is, of course, what everybody is searching for as we have this rising tide of chronic disease.

Identifying Epigenetically Labile Targets Difficult Due to Species Variability
RJ: Right, but the problem right now is that we don't really know what the epigenetically labile targets are. There are going to be a lot of them probably, but there are probably some that are going to be much more important than other ones. This, I think, brings in a big issue here, and that is that the species vary in their epigenetically labile targets. They've got to because if we have software that is telling how an individual species develops, and that's the programming that is going on, and it's not really totally the genes that are involved, but how they are regulated. You have to have different programs running in a mouse, for example, versus a human.

With bisphenol A, we showed (and I think other people too) that it reduces the methylation that one normally sees, at least in the model system we used. And in that system we get all of these different effects: yellow animals, obese, diabetes, all that type of thing. But I can't really even extrapolate that to another strain of mice because other strains of mice don't have that transposable element-that little viral set of DNA-upstream of the Agouti gene. What I think we can extrapolate is that this tends to cause a reduced ability to methylate. But what effects it has and what the targets are that it is effecting will vary-I believe-between species. That is going to make extrapolation more difficult between species. This is important because we use animals as surrogates for humans to determine treatments and also to determine what compounds are problematic (risk assessment).

JB: Wow, that's a very insightful comment. Again, we could go on and on. I guess the best thing I can say right now is if the environment we are in influences our epigenome in positive or negative ways, then these minutes that you have spent with me have definitely created a healthy epigenome for me. This conversation is sending the right messages to my epigenome. So, thank you very, very much. Your continued diligence, and leadership, and very articulate and what I would say "news-to-use"-type of approach to this very complex topic is really refreshing. I really appreciate you spending the time with us.

RJ: Well, thank you very much for talking to me. I do love this field of research. It is gratifying to see the field grow as rapidly as it is growing. I was asked one time about the field, and it looks, right now, like the papers that are being published in this area are doubling every one-and-a-half to two years. We're in the vertical phase, basically, of the epigenetic rise, which means the present, past, and future are merging. What does this mean? It means it is very difficult to predict what's going to happen.

JB: It is my deep hope that when the awards are given for the first pioneers that brought this concept into

the 21st century vision that the name Randy Jirtle will be at the head of the list because you certainly deserve it and we thank you very much.

RJ: You're way too kind. Thank you very much

Bibliography

1 Attig L, Gabory A, Junien C. Nutritional developmental epigenomics: immediate and long-lasting effects. Proc Nutr Soc. 2010;69(2):221-231.

2 Doolin MT, Barbaux S, McDonnell M, et al. Maternal genetics effects, exerted by genes involved in homocysteine remethylation, influence the risk of spina bifida. Am J Hum Genet. 2002;71(5):1222-1226.

3 Chiam K, Tilley WD, Butler LM, Bianco-Miotto T. The dynamic and static modification of the epigenome by hormones: a role in the developmental origin of hormone related cancers. Biochim Biophys Acta. 2009;1795(2):104-109.

4 Barth TK, Imhof A. Fast signals and slow marks: the dynamics of histone modifications. Trends Biochem Sci. 2010 Aug 2. [Epub ahead of print]

5 Wallace DC. Bioenergetics and the epigenome: interface between the environment and genes in common diseases. Dev Disabil Res Rev. 2010;16(2):114-119.

6 Beach RS, Gershwin ME, Hurley LS. Gestational zinc deprivation in mice: persistence of immunodeficiency for three generations. Science. 1982;218(4571):469-471.

7 Hanwell HE, Banwell B. Assessment of evidence for a protective role of vitamin D in multiple sclerosis. Biochim Biophys Acta. 2010 Jul 30. [Epub ahead of print]

8 Cloud J. Why your DNA isn't your destiny. Time. 2010 Jan 6.

9 Bernal AJ, Jirtle RL. Epigenomic disruption: the effects of early developmental exposures. Birth Defects Res A Clin Mol Teratol. 2010 Jun 21. [Epub ahead of print]

10 Dolinoy DC, Huang D, Jirtle RL. Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. Proc Natl Sci U S A. 2007;104(32):13056-13061.

11 King MC, Marks JH, Mandell JB. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science. 2003;302(5645):643-646.

12 Kantarjian H, Issa JP, Rosenfeld CS, et al. Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study. Cancer. 2006;106(8):1794-1803.

13 Skinner MK, Guerrero-Bosagna C. Environmental signals and transgenerational epigenetics.

Epigenomics. 2009;1(1):111-117.

14 Szyf M. The early life environment and the epigenome. Biochim Biophys Acta. 2009;1790(9):878-885.p>