March 2009 Issue | Kevin V. Morris, PhD Assistant Professor

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Welcome to *Functional Medicine Update* for March 2009. Functional medicine, like all other disciplines within the health sciences, starts with good observation. What does the patient tell us about the nature of the intervention? What does the patient tell us about their interaction with their environment and how that presents itself into their phenotype or their health patterns? These are the kinds of questions that challenge every health practitioner no matter what their discipline, and have historically been the challenges that have confronted the development and evolution of making a more efficacious, more outcome focused, healthcare delivery system.

New Therapeutics Are Dependent on Information Being Developed at the Cellular Level

In this issue of *Functional Medicine Update*, we are going to have the opportunity to speak with a person whose literature you (maybe) would not come in touch with if you are a clinician because it appears very, very esoteric relative to the daily practice of health care. Articles with titles like "Small Interfering RNA-induced Transcriptional Gene Silencing in Human Cells" don't seem like the kinds of things you'd probably include in your everyday reading.[1] But as you will learn over the course of this issue of *Functional Medicine Update*, what we are starting to recognize through articles by investigators like Dr. Kevin Morris from the Scripps Institute, is that the nature of the decisions that we make in the clinic--the observations we make and how they are sieved into new therapeutics--are dependent upon new information being developed at the cellular level in understanding how the environment influences gene expression.

One of those areas, which we will learn in more detail in this issue, relates to what are called small interfering RNAs (ribonucleic acids). RNAs can exist in a variety of different forms in the cell: they can be messenger RNA, they can be ribosomal RNA, or they can be related to transfer RNA. Each one of those RNA forms has a very important role to play in the transcriptional process of taking information from outside the cell and converting it into inside information, in terms of the proteomics of the cell converting that information into specific proteins and enzymes that ultimately regulate the function of the cell, tissue, organ, or organ system.

What we are starting to witness is the fact that these epigenetic factors (the factors that occur within the scope of not changing the genes in and of themselves but regulating the expression of the genes at promoter regions and transcriptional element control) are, in part, controlled not only by things we've discussed such as methylation, acetylation, phosphorylation, and ubiquinitation, but also by the appearance in the cell of these small interfering RNAs (now recognized to be in the thousands). These

RNAs were previously thought to be kind of junk, but are now recognized to have very critical roles to play, epigenetically, in modulating how cells function, day to day, and respond to their environment. This recognition ultimately weaves itself into a better understanding of the individual response a patient has as we evolve a personalized medicine approach that includes looking at the symptoms, signs, antecedents, history, and genotypic variables within a patient start to better understand the complex nature of their chronic illness

Let's take that high-brow, blue-sky introduction down to the ground level of clinical observation. Over the course of years, we all probably have thousands of anecdotal stories from practice. This anecdote is about a 19-year-old young man that came in to visit us who had had cystic acne for many years. From the age of about 14 on, he had had this disfiguring acne. He reported that in junior high school and then later in high school there was always at least one kid whose face was worse than his, but by the time he got to college it looked like he had the worst skin and he was very, very embarrassed about it. His skin attached a stigma to him relative to his socialization. He said he made absolutely no friends and he was too self-conscious to approach anyone. He would stay up late at night so he could do lengthy skincare routines when his roommates were asleep. He took virtually everything, and had visited dermatologists/specialists to try to find answers to this question. He had been to a number of doctors and had been unsuccessful with treatments, including taking Accutane. He eventually found his way into one of our functional medicine research projects at the Functional Medicine Research Clinic in Gig Harbor, WA, and was evaluated for his relative history.

Could Skin Problems Originate in the Gut?
Asking different questions can often get different answers. As you know, a functional medicine provider often throws a broader net in looking at the ecology of an illness, the home of where that thing might have originated. In discussing this situation with this young man, it turned out that he had a considerable amount of digestive problems in his history, which he had never really been asked about. The question was: could the origin of his skin problem have originated by immunological dysfunction in the gut? This was a fairly simple hypothesis, but one that had never been asked before.

The concept was that maybe this patient needed to be put on a 4R-type intervention program. "Remove," "Replace," "Reinoculate," and "Repair" are the "4Rs" of the gastrointestinal restoration program. The first "R" involves removing the foreign agents that might be altering the immune system (this could be antigens, allergens, toxins, or heavy metals). "Replace" means to replace digestive enzymes and/or bile acids as necessary. The third "R" is "Reinoculate," which has to do with the pre- and probiotic supplementation to reinoculate the gut with friendly symbiotic bacteria. And then the fourth "R" is "Repair," which refers to the addition of nutrients that are necessary for support of proper mucosal repair (pantothenic acid, zinc in a non-irritating form, the amino acid arginine, vitamin E, and essential fatty acids).

With that as a strategy, he was put on a probiotic high potency along with a prebiotic daily containing arabinogalactans and fructooligosaccharides and non-fermentable carbohydrate. The combination of the prebiotic and probiotic was accompanied by nutritional supplementation with omega-3 fatty acids in an enterically coated form (about 3 grams a day), and good support with regard to the nutrients necessary to support mucosal repair. His diet was also evaluated to see if it contained potential antigenic or allergic substances. It didn't appear as if that was a major offender in his case. According to blood tests, there didn't appear to be any major sign of an IgG- or IgE-related problem, so his diet was modified very little

(in fact, I would probably say none at all). Yet on the addition of the prebiotics, the probiotics, and the other nutrients, he had a remarkable response within a period of just a few weeks.

What was his response to the program? I am paraphrasing his words: "About three days after starting the program I realized that my skin looked much less red and that it seemed some of my cysts that I had had for six years seemed to be calming down. Everyday I thought my face looked a little better. It has been now over three months and for the first time in the last nine years I don't have acne. I couldn't believe that I wasted so much time trying different types of treatments and seeking different doctors' opinions who couldn't do anything for me when all I had to do was just to consume the appropriate nutrition."

This is an example of taking a very complex etiology and condensing it down to a fairly simple intervention using a functional medicine conceptual framework. You might ask: what was going on in his environment (the local environment) that would have created an immunological problem that was seen as a symptom of this acne vulgaris? I don't think we can answer all those questions. I think that's probably a very complicated set of associations. I do think we can postulate that this complex relationship that we have with our gastrointestinal-associated lymphoid tissue--how it picks up information from the gut, and how those messages are translated through our enteric bacteria ultimately into our GALT--is becoming a more well understood component that relates to this environmental connection to our health.

I believe that this is a very important takeaway from what you are going to be hearing about with regard to molecular genetics and cellular biology around small interfering RNAs. What is it that the environment does that then modifies the cellular milieu in such a way as to create different expression patterns of our genes and ultimately, then, lock us in to these cycles of chronic illness for which we then take therapeutic agents to treat the effect, not ever having really appropriately identified nor treated the cause?

Let me give you another example of this that goes back to our discussion with Dr. Michael Skinner from Washington State University. We talked about environmental epigenetics with Dr. Skinner, and about the brilliant work he has been doing in looking at the role that low-level environmental chemicals can have on epigenetic marks and transmission effects through the generations of these alterations in epigenetic signals. An interesting paper was just published in the January 28, 2009 issue of *Human Reproduction Advances* titled "Maternal Levels of Perfluorinated Chemicals and Subfecundity."[2] I think this paper is kind of another step in understanding this emerging frontier of how environment plays roles in modulating function through conception, fetal development, and infancy. In this particular study, the plasma levels of perfluorinated chemicals were evaluated at weeks 4 – 14 of pregnancy among 1240 women from the Danish National Birth Cohort that were recruited from 1996 to 2002. For this pregnancy, women reported time to pregnancy in five categories. Infertility was defined as having a time-to-pregnancy of greater than 12 months, or receiving infertility treatment to establish pregnancy.

What was found from the study? There was a very strong association between longer time to pregnancy and higher maternal levels of the perfluorinated chemicals and also the relationship it had to infertility. Compared with women in the lowest exposure quartile, the adjusted odds of infertility increased by 70 to 134 percent among women in the higher three quartiles. Fecundity odds ratios were also estimated using so-called Cox statistic discrete time models, and it was really found that there was a high impact of the exposure of these chemicals and body burden of them on lowered ability to conceive and have children. The findings suggest strongly that exposure to this class of chemicals, and exposure to them for some period of time with increasing plasma levels, in the general population reduces the ability to conceive.

These exposure levels are very common in developed countries, which may allow some explanation for the rising incidence of infertility among couples.

This all goes back to the question: are we putting epigenetic marks on our book of life in such a way as to alter the expression of processes that are related to conception and to appropriate term births and deliveries? These are subtle questions that talk about our understanding at cellular levels as to how these signaling processes actually occur. Again I'll come back to our discussion about small interfering RNAs because we might say, "Well, these are part of the story. These are part of what's going on in the cell that can mask or uncover specific regions of our genome that are modified by the environment that create different expression patterns."

Managing Early-Stage Symptoms of Cognitive Decline

Let's take another example. I think this is another interesting emerging story, and that is the story of dementia-let's call it cognitive decline in older age and then later Alzheimer's dementia. We are all on the search for the Holy Grail: what is it that causes Alzheimer's disease and leads to these neurofibrillary tangles and the beta-amyloid deposition and the loss of hippocampal function that we ultimately associate with memory loss and Alzheimer's disease? What can we do to prevent this from occurring, and what are the therapeutic agents that might be useful in managing early stage symptoms of cognitive decline (this period where we might do what is called neuroprotective therapy, where you can intervene early and prevent the course of continued loss of cognitive decline)?

In order to answer that question, one might ask this question first: is Alzheimer's disease strictly a genetic disorder? If we go to the literature and look at the genetics of Alzheimer's, there are certainly certain gene loci (like the apoE4 genes) that have a strong linkage, but I don't believe there is any data to indicate that Alzheimer's dementia is a homozygous-type of tight-gene-linked genetic disease. It rather is one of those disorders associated with the altered function or expression of many genes that ultimately regulate neurological function. It is one of those disorders that we would tie together with genetic uniqueness and environmental exposure.

When we start looking at various types of environmental exposures or environmental features that might associate themselves with either the protection against or the increased prevalence of Alzheimer's disease, we are obviously led to questions surrounding the diet. In an earlier issue of Functional Medicine Update we discussed this paper by Dr. Mattson at the NIH on neurohormetic chemicals that are found in our diet. Neurohormetic chemicals are actually phytochemicals (food-derived materials) which he postulated (from animal studies) might have a positive impact on the prevention of Alzheimer's-like etiology through the emerging mechanism of productions of these neurofibrillary tangles.3 These phytochemicals-these neurohormetic protective phytochemicals-include things like sulfuraphane from cruciferous vegetables, and EGCG found in green tea, things that were related to red wine and peanut skins, like resveratrol polyphenols, and alpha-acids that come from various foods that relate to the root vegetable family. These, he was suggesting, are chemicals that in animal models, when the animals are dosed with higher intake of these in their diets, were found to actually slow the rate of what might be considered the animal model of Alzheimer's dementia.

It raises a question as to whether diet might play a role in both the prevention and even the relative prevalence because the alternative of a diet rich in phytochemicals is a diet that is devoid of

phytochemicals and rich in sugars and fats and other types of neurotoxic substances that activate the NMDA receptor sites and increase glutamate transport in the brain and increase oxidative mitochondrial injury. These would be foods that are associated with what we call type 3 diabetes, which is the new term that has been associated with the blood sugar insulin dysfunction that we think is an etiological agent in Alzheimer's disease. This was also a topic that we have discussed in previous issues of Functional Medicine Update. The emerging literature is supporting the fact that dysinsulinism is not only associated with diabetes and cardiovascular disease (breast, prostate, and colon cancer), but it is also associated with Alzheimer's dementia through this type 3 diabetes.

If we were to ask, then, "Has our diet been moving in the way that the environmental signals that come from it are signaling through cellular receptor systems two different things? Number one is reducing the neuroprotective chemicals that are within a complex minimally processed whole foods diet, and number two is increasing the dysfunction of insulin and other signaling molecules that activate neuronal oxidative injury and apoptosis," the answer could likely be 'yes.' That is the way our culture has drifted, and therefore it is not just that we are getting older, alone, that is the explanation for an increasing prevalence of age-related dementias, but it is also getting older in a suboptimal environment: setting dysfunctional signals in our neurons.

With all of that as an introduction, let's talk about three interesting papers that were just recently published. The first is a paper that appeared in the Archives of Neurology very recently in 2009 that describes the Mediterranean diet and its relationship to mild cognitive impairment.4 Having spoken to this concept of the low glycemic load Mediterranean diet for some time within Functional Medicine Update, I think we all recognize this is a diet that contains considerable amount of phytochemicals through the colored foods and the minimally processed foods that are found in the traditional Mediterranean diet. The Mediterranean region is a very diverse region with many different diets that really fall within that theater of influence, but that there are characteristics that tie these diets together. Meat is generally considered kind of a secondary component of the diet. Fish is eaten more frequently than in America. There are a lot of colored, minimally processed vegetables. Fruits are consumed. These are generally what we could consider to be more organic. There is a lot of olive oil (generally first-pressed type olive oil with a lot of rich phytochemicals in it), and of course there is the wine component as well, with one to two glasses of wine a day often consumed. So you have all these characteristics-a type of pattern of eating-that is low-glycemic load, complex and phytochemically rich.

What is this article that appeared in the Archives of Neurology in 2009 say to this? These researchers looked at an ethnic community and evaluated people that were consuming the Mediterranean diet by choice (this happened to be a community in New York, not in Sardinia, or Corsica, or southern Italy). These were people of Mediterranean extraction who continued to consume their traditional diet, but were living within New York, so you can't just say this is solely a consequence of living in a different place in the world. They were in a very high-density urban environment. The researchers looked at things like age, sex, ethnicity, education, apoE genotype, caloric intake, and body mass index as variables. In this prognostic study, they followed 1393 cognitively normal participants over an average period of time of about four and a half years (the range was from 1 to 16 years), of whom 275 developed mild cognitive impairment. They then looked at those individuals who had very high adherence to the Mediterranean diet versus those who had low adherence (they broke them into cohorts). The results were quite stunning. The data separates itself out with a high statistical significance, and they found that the higher the adherence to the Mediterranean diet, the more association there was with a trend for reduced risk of developing mild

cognitive impairment and a reduced risk of the conversion of mild cognitive impairment to Alzheimer's disease. There was a pretty dramatic separation of the two data sets.

Obviously this is an association study. I want to be very cautious that we don't go from association to proving causality. Other studies that have been published, for instance, a previous study looked at the Mediterranean diet and the risk for Alzheimer's disease and was published in the Annals of Neurology.5 This study looked at a community of 2258 non-demented individuals (again in New York), who were evaluated every one and a half years for their cognitive performance. It was found that of those who adhered to a Mediterranean diet, it was the main predictor of all the variables they looked at, including apoE genotype, for determining the trajectory towards whether they were going to get Alzheimer's disease in later life or not. In fact, it was more powerful a predictor than apoE genotype.Those who adhered very strongly to a Mediterranean diet over the period of time of study had a much lower incidence (in fact about
80{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} lower risk) of Alzheimer's disease versus those that were in the lowest tertile of compliance with a Mediterranean diet.

Again, we ask, how could this actually work? What are the values of a Mediterranean-style diet and its relationship to function of the brain? To fully answer that question it is going to take years of research. We have to look all these signaling processes of how the various components of the diet would work their way up from the gut, to the plasma, to neurochemical signaling, and ultimately we look at glial and neuronal function. I mean, it is going to be a fascinating exploration into this connection between environment and neurological function. But we can say, I think, at the whole-organism, kind of broad-brush level, that what's going on is that the Mediterranean-style diet is a lower glycemic load. It stabilizes insulin levels; it has lower postprandial blood glucose effects.
We know those people have lowered hemoglobin A1C, or glycosylated hemoglobin levels, so you are having less glycation of proteins. Less inflammatory biomarkers are produced, hsCRP levels are lower, and as a consequence of all these immunological effects -- cooling off the immune system, and lowering inflammatory potential-- and favorable effects on glial cell function (knowing that the glial cells are kind of brethren, or close relatives, of the macrophages, and the Kupffer cells in the liver and the GALT cells in our gastrointestinal tract), that that communications system is kind of put at rest. And so our model would be the combination of the phytochemicals that modulate function of the signaling process in conjunction with the lowered glycemic load, results in a very dramatic effect, over time, on neurological function. And then you lay that on top of genetic susceptibility factors and it expresses itself finally as this reduced incidence of Alzheimer's disease.

These are very profound new chapters that are being written as it relates to how, at the molecular, cellular, and tissue, organ, and organ system level, that actually our environment can influence a very large constellation of diseases, including things like Alzheimer's dementia, which some people have just kind of relegated to say, "Well, it's because we're getting older and everybody will get Alzheimer's eventually, and those with the most genetic susceptibility get it first." That's a very simple deterministic model that I think neglects all this other emerging literature that is really coming to light.

Even things like mid-life coffee and tea drinking have been found to influence the risk of late-life dementia. A recent paper was published in 2009 that I thought was quite fascinating, out of the department of neurology in Kuopio, Finland.6 This group of researchers looked at the role that caffeine has on central nervous stimulation and also some of the other flavonoids that are found in tea and coffee,

and other phytochemicals, and how they modulate function. They looked at the consumption of coffee and tea drinking and its relationship with various types of Alzheimer's disease risk factors, including apoE4 genotype and depressive symptoms and other lifestyle and vascular factors.

According to their data, the lowest risk (in fact, 65{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} decreased incidence of Alzheimer's disease) was found in people who drank 3 to 5 cups per day of coffee. Tea drinking was relatively uncommon and was not associated with dementia or Alzheimer's disease. Drinking these phytochemically-rich beverages at midlife was associated with a decreased risk of dementia later in life. The researchers go on to say this finding might open possibilities for the prevention of dementia.

Again, this is just part of the story. It is not that we're going to have a magic bullet: just increase your consumption of tea and coffee 3 to 5 cups a day and there will be no more Alzheimer's. What we are saying is that there are many different contributions to a phytochemical matrix that might have neurohormetic effects in lowering the incidence and risk of the mode of action, or the etiology, of Alzheimer's disease. The combination of the Mediterranean diet composite and how that signature of nutrients influences gene expression as well as these other phytochemicals that are found in tea and coffee- a different story is emerging. By the way, this was in the in the Journal of Alzheimer's Disease, the first volume of 2009, if you want to follow-up on this mid-life coffee and tea-drinking study and the risk to late-age dementia. Again, diet is a very complex modulator of environmental signals that has influence, then, on things like small interfering RNAs, and methylation patterns, and phosphorylation patterns, and acetylation patterns of the genome and how that translates itself ultimately, epigenetically and nutrigenomically, into messages that then wash their way through a person's genetic uniqueness into their phenotype and how they specifically look, act, and feel.

With all of that as kind of a presage, you probably know that there was a study that was just published in the Archives of Internal Medicine in 2009 titled "Multivitamin Use and the Risk of Cancer and Cardiovascular Disease in the Women's Health Initiative Cohorts."7 This was a study done at the Fred Hutchinson Cancer Research Center at the University Washington School of Medicine in Seattle, WA. I think it is a very interesting study. This study included 161,808 participants from the Women's Health Initiative clinical trials. These were 68,000 women in three overlapping trials of hormone therapy, diet modification, and calcium and vitamin D supplements. What they did is collect detailed information on multivitamin use, baseline and follow-up time points, and the study enrollment was between 1993 and 1998. A total of 41.5{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} of the participants used multivitamins, so it was a fairly good penetration of individuals who self-selected to supplement daily with multivitamins. After a median of 8 years of follow-up in the clinical trial cohort and about 8 years in the observational study, there were 9619 cases of breast, colorectal, endometrial, renal, bladder, stomach, lung, or ovarian cancer; and 8751 cardiovascular events; and 9865 deaths.

They then looked at multivariate-adjusted analyses to see if they could tease out whether those individuals that took multivitamins daily had any difference in incidence of any of these health conditions versus those that did not. They came to the conclusion that there was no impact on any of the parameters measured (in any of the diseases measured) by taking multivitamins. They went on to say, after a median follow-up of 8 years in the clinical trial and observational study cohorts in this Womens' Health Initiative group, there was no convincing evidence that multivitamin use had influence on the risk of common cancers, cardiovascular disease, or total mortality in postmenopausal women.

Here is another study in the kind of litany of studies that have been published recently that tends to argue that vitamin supplementation doesn't have any positive benefit. So what can we say about that? There are a number of features which keep coming back to the story that I have been developing over the last several years about these intervention trials (this is actually an epidemiological trial-self-supplemented epidemiological trial, so it is not a true cohort-controlled, intervention-type of blinded trial, but, again, it tends to suggest that supplements don't have any long-term health benefit).

The model that I'm trying to get people to understand is a model that may be very different than that which was espoused some 25 years ago in the book Life Extension.8 Some of you remember that book by Pearson and Shaw, in which they propounded that as long as you took an appropriate number of supplements you could neutralize the effects of any bad diet or bad lifestyle; it was all about taking very large amounts of supplements to kind of get the best of all worlds against whatever decisions you might make in your diet and lifestyle.

At that point in time, 25 years ago, I made a very strong statement on Functional Medicine Update that I felt this was going to be proven to be inappropriate, that supplementary nutrients were not the primary feature that modified our function, but rather we had to consider our lifestyle variable first. We had to be on an appropriate diet, we had to consider our stress and our exercise, environmental pollution, smoking, all these things were very big tuning knobs for influencing our health outcomes that we couldn't just neutralize easily by taking doses of multivitamins. But for those places where there are nutrient gaps in which specific kinds of nutrients like B6, or calcium, or iron, or antioxidants like vitamin E might be in less than optimal levels, the insurance policy of a good multivitamin/mineral high-potency bioavailable formula would be helpful in filling in those nutrient gaps. It would be kind of the combination of dietary, lifestyle, and nutritional supplement on a preventive feature that would be proven valuable.

Well unfortunately, these aren't the kinds of studies that are generally done. We don't control the diet and lifestyle of these individuals, we just take the luck of the draw and then we say the vitamins didn't work. Sometimes the signals of the supplements are lower intensity than the adverse signals of a poor-quality lifestyle and diet (it swamps that information). If you did cohort analysis within the full data set, you might find people who really regulated their lifestyles very well, and by the addition of supplements they got added advantage, but they would be kind of washed out of the data set by all the kind of "luck of the draw" individuals that were just eating whatever they want and taking a vitamin supplement, hoping that that is going to be the insurance policy.

I think what we are emerging is to recognize it is the composite signals that come from our lifestyle and our environment, our diet, that then give rise to the expression of our genes and puts epigenetic marks on our genes and modulates things like the small interfering RNAs that may be affecting, then, our gene transcription processes and regulating both somatic and germ cells. We also recognize that this environmental toxicology issue is not inconsequential, starting at the moment of conception and moving through our whole life, and how it influences cellular function.

My thought about this recent published paper in the Archives of Internal Medicine is that it is not really a big surprise that there was no direct link upon reduction of cardiovascular incidence or cancer incidence in a large cohort of people who are doing the common things that we see our society doing, that is, eating high-calorie, low-nutrient density, poor-quality depleted foods and having the sense that maybe they can, by taking a multivitamin, ensure good health in the absence of making good decisions. It's really just kind

of once again affirms the complex nature of how we are influenced by the signals in our environment.

The Council for Responsible Nutrition, which is an organization, as you know, that represents the science of the nutritional supplement industry, released a press release in response in response to the Archives of Internal Medicine article.9 In this press release, they say that multivitamins, like all other dietary supplements, are meant to be used as part of an overall healthy life and are not intended to be magic bullets that will assure the prevention of chronic diseases like cancer. As I have already said, the key to good health is a commitment to an overall wellness approach that includes the daily use of a multivitamins, and that's also the position of the Council for Responsible Nutrition.

With that in mind, you might say, "Is there a difference between nutritional prophylaxis that would be, say, generally everyday maintenance of a good, healthy set of signals going to your genes, and that of therapeutics? Is there a place for nutritional pharmacology?" That term was first used by Dr. Spiller in the book titled Nutritional Pharmacology that he authored back in the late 1980s.10 I think that there are, obviously, places where we would use therapeutic doses of specific nutrients to get beneficial effects, like the vitamin D story that is starting to emerge.

Continuing Discussions on Vitamin A and D Supplementation

I was very pleased that one of our long-term *Functional Medicine Update* subscribers, Dr. Warren Levin, who has been a very noteworthy expert in this field for more than four decades, shared with me a paper that he had received back in the 1970s from Dr. Arthur Alexander Knapp, who was practicing medicine in New York City and had an idea to use higher therapeutic doses of vitamins A and D in patients that had various types of ocular and ophthalmological problems, including myopia, and was able to publish work that is very interesting.[11] This paper Dr. Levin sent me authored by Dr. Knapp looks at 40 years of his research with the supplemental dose of nutrients in people that had certain ophthalmologic problems, showing that supplementation had a very distinctive benefit in helping protect against things like macular degeneration and also improve eyesight beyond that just of wearing glasses, that there was improvement, overall, in vision.

There is a difference between nutritional therapeutics and nutritional preventives. That leads to some interesting recent papers that just appeared in the literature around this vitamin D and vitamin A story. There is a very nice paper on cod liver oil, vitamin A, respiratory infections, and vitamin D that was coauthored by Reinhold Vieth and Walter Willett, and really a whole list of individuals, all of whom have all been looking at the association between vitamin D and immunological problems.[12] What they find in this paper and suggest is that omega-3 fatty acids and vitamin E coming through cod liver oil historically used by our grandparents or great-grandparents for tonics have very dramatic effects on improving immune function in children and lowering the risk to frequent respiratory infections, so maybe there is something very interesting about the flu, for instance, and relative risk to the flu as a consequence of vitamin D and vitamin A status.

There actually is a very nice review on the epidemiology of influenza that was just reported that actually discusses this whole connection to vitamin D status and increasing incidence of infection with the flu. This appeared in the *Virology Journal* in 2008.[13] The authors of this article point out that the epidemiology of influenza swarms with incongruities, but one of the things that seems to be central is that

many times epidemics of the flu are associated with periods of either nutritional insufficiency or poor solar radiation, giving rise to lowered vitamin D associations and potential increasing risk of infection. They go on to say that we ought to be looking at the vitamin D connection to influenza, and that adequate vitamin D levels may be very important along with vitamin A for support of the immune system and defense against viral infections.

Similarly, as you probably know, we're seeing more and more evidence come out supporting the fact that vitamin D may be very helpful for things like immune inflammatory disorders, like multiple sclerosis (MS), which appears to have a susceptibility gene that is a vitamin D metabolite, 1,25-dihydroxycholecalciferol-responsive gene. In certain people who have this genetic risk, they require higher levels of vitamin D in order to promote appropriate function.[14]

What are we really speaking about? We are speaking about the nature of individuals who have expressed risk that is different than other people, that is encoded within their genes, and their environment is going to be unique to them, and the response to it is going to be unique to their gene sieving process. That particular translation process occurs through both epigenetic and genetic mechanisms, and we are going to be very fortunate to hear a little bit about what this emerging story is around small interfering RNAs and how they are another part of this complex milieu within the cell that translates messages into gene expression patterns. Let's now share this story with our researcher of the month, Dr. Kevin Morris.

INTERVIEW TRANSCRIPT

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We can hardly wait to get to our clinician or researcher of the month every month because we know that's where the cutting edge is going to be exposed. That's where we're going to look at where the field is going. That's where we'll find new tools and new opportunities to contextualize that private moment in the exam room with a patient and ask, "How did they get here? What is the etiology of their condition and how might I frame it in a different way to get improved outcomes?" You are not going to be disappointed this month. I'm very fortunate that we are going to have Dr. Kevin Morris as our expert. Let me just contextualize this because it may be a little bit of a stretch for some of you, but I'm asking your patience and your indulgence because I think you are going to be very pleased to see the outcome. You might even be surprised at how far you'll come in this discussion in understanding how much you really already know.

Dr. Morris is a leader in the field of what I guess you would call post-translational/post-transcriptional gene silencing (or TSG, transcriptional gene silencing). This really falls in to the scheme of things that

you have heard about on Functional Medicine Update over the last year, which had to do with epigenetics and how the gene expression is modulated through the environmental factors, both intracellularly and extracellularly. We have had Dr. Michael Fenech from CSIRO in Adelaide, New Zealand talk about genomic instability. We had Dr. Randy Jirtle talk about nutritional epigenomics from his work at Duke. We've had Dr. Michael Skinner talk about environmental epigenomics and genetics from his work at Washington State University, and Dr. Edward Calabrese talk about hormesis and the effects that small concentrations of agents can have on modulating cellular function.You are developing kind of a comfort level with the language of where this field is going, and you're going to take another very important step today with Dr. Kevin Morris.

Let me give you a little bit of a background on Kevin. He has had very, very rich productivity for an investigator. I hope it is not sounding pejorative, but I would call him a young investigator (in that anybody less than 60 years of age these days I call young). He started off at the University of California at Davis, and after his PhD went on to a postdoctoral fellowship at UCSD. From there he went to the Beckman Research Institute at the City of Hope in Duarte, CA, where actually he was the co-author/principal author on a paper that appeared in Science magazine in 2004 titled "Small Interfering RNA-induced Transcriptional Gene Silencing in Human Cells," which turned out to be, I think, a very pivotal discovery in that up to that point (as far as I know) there had not been the proof that the small RNAs could silence genes in mammalian cells. So this was kind of an interesting step that you are going to hear more about from Dr. Morris.

He has now achieved, I think, very notable distinction as an Associate Professor in the Department of Molecular and Experimental Medicine at the Scripps Institute in La Jolla, CA, where he oversees post-doctoral students and is engaged in very active studies. Last year-2008-was a very productive year for him and his group, with some very important published studies coming out that help us to understand this post-transcriptional gene regulation through these interfering RNAs. With all of that as kind of a very complex introduction, Dr. Morris, welcome to Functional Medicine Update. It is really a pleasure to have you as an expert on our audio magazine.

KM:Thank you, Jeff. It's a pleasure for me to be here, as well, and be honored with an interview and discuss my work further with the medical community.

JB:We are dealing, here, principally with clinicians, who might say, "What in the heck are small interfering RNAs, or what are micro RNAs, and from that, what do they have to do with medicine?" Maybe we should start with the basics, here. Maybe you could just describe the whole concept of micro RNAs and small interfering RNAs and what we have learned because it is a fairly new concept, really, within probably the last decade that has become more understood.

Definition of Small Interfering RNA

KM:Right. Initially I'll just go into a brief summary of the history. In 1987, some folks doing plant genetics found that small double-stranded RNAs could turn off a gene (the expression of the mRNA). As we know in basic biology, your DNA makes mRNA, which then makes a protein. And they found, in plants, that these small interfering RNAs could direct transcriptional gene silencing. What that means is that these small interfering RNAs are double-stranded small RNAs that are 21 to 27 nucleotides long and they are double-stranded pairs. They enter into a complex in plants and they match the corresponding DNA of the gene promoter, and what they do is they cause epigenetic changes at that promoter that lead

to downstream silencing of that gene. The important part of transcriptional silencing in small interfering RNAs is that it is inheritable-it is passed on to daughter cells.

siRNAs and Post-Transcriptional Gene Silencing
In the late 80s they figured out that this was occurring in plants and that it has been shown in yeast. In 1998, Andy Fire and Craig Mello showed that small interfering RNAs could also direct post-transcriptional silencing, and what that is these small RNAs target the transcript that is making the protein and they cut it. And then in 2004, my lab was able to show that you could take these small RNAs and design them-knowing the sequence of a gene promoter-design them, make them, in the lab and put them into cells, and that they would go to that promoter and turn that promoter off in human cells.

With small interfering RNAs, I think what opens up the whole medical area is that we can design them a priority, knowing the sequence of whatever we want to target. We can target a gene either post-transcriptionally (like Andy Fire and Craig Mello did and they received the Nobel Prize for this in 2006) and that cuts the transcript-it cuts it and it no longer makes a protein, however, you still get the transcript being made, and over time you'll regain the expression of that gene. Or you can target it transcriptionally, which is the way my lab does it. You target the gene promoter, and that works mechanistically through a different sort of paradigm. In that paradigm, it causes epigenetic changes: histone methylation and DNA methylation at the gene promoter, and that causes that promoter to be less accessible to the transcription machinery, and it can result in long-term silencing, where the gene is turned down in a more permanent manner.

Now, we design these small RNAs knowing the sequence that we are targeting, but up until this point it was not known whether this was an endogenous mechanism in human cells. We knew that we could do this (we could put the small RNAs into the cells), and we knew the machinery was there to turn off the gene transcriptionally, but we didn't know whether there were RNAs doing this endogenously. There have been microRNAs that have been reported, and piRNAs-other different species of small RNAs have been reported-but none of them have been shown to work to transcriptionally turn off a gene. It was in 2008, where we recently published, that we found that noncoding antisense, which make up (presumably) 25{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} of all genes that are being expressed can actually do this.

JB:First of all, congratulations on summarizing a huge body of information in a very concise way. You have obviously said that before; that was very, very well done. And secondly, this kind of little haiku-this little pearl-that you dropped at the end, which is in 2008, your work and that of others has now defined that these small RNAs are produced endogenously and can serve as post-translational modulators and that that constitutes maybe 25{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} of expression patterns, is a pretty profound concept, it would seem to me.

What Regulates the Production of RNA to Begin With?
KM:Let me clarify that. There are microRNAs being made, and they are post-transcriptional modifiers; they inhibit the mRNA from being made into a protein. But what I am talking about is even further upstream of that and saying, "Can we even stop the mRNA? What regulates the production of the RNA to begin with?" So to think about it in terms of snow on a mountain, we want to stop the snow from even falling onto the mountain as opposed to melting and flowing into a river, etc. The post-transcriptional targeting...it's like damming up a river; it is stopping the water flow that way, whereas transcriptional

targeting is stopping the snow and rain from even occurring.

And so what we work on is the transcriptional targeting, which is upstream in the gene promoter. What has not been known was what is controlling this. Are there RNA species controlling this? What we found out is that there are non-coding RNAs that do this. I think it is 1.2{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} of the entire human genome is making proteins. That leaves about 98{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} of the genome with what we traditionally used to call "junk" DNA. Nobody knew what that was doing and some people speculated that it was involved in spacing the genes out so they could be expressed and controlled, etc., and there may be some aspect that the DNA does that. But it turns out that a lot of that junk DNA is actually made into RNA, and nobody has understood what those RNAs are doing, and those are called non-coding RNAs; they don't make a protein. What we showed in 2008 in this PLoS Genetics paper is that there are non-coding RNAs that can regulate the gene's transcription.15 That was sort of a big "a-ha" moment, where it is like, "Oh, small RNAs can do this, too." And that is how we found that we could even do this in human cells. Now we are actually on the tip of the iceberg, figuring out the RNAs that are inside our cells that are doing this.

The interesting thing to point out is that several of these non-coding antisense RNAs have been documented in tumor suppressor genes, and tumor suppressor genes tend to (in cancers) become epigenetically silenced, and so that fits in with this whole discovery where the non-coding RNA is involved in this regulation. The notion is what happens over time is that these non-coding RNAs become uncontrolled or overexpressed, and they lead to silencing of the promoter for, let's say, a tumor suppressor gene, and that tumor suppressor gene gets silenced and that can be sort of the epigenetic prelude to developing into a cancer sort of state. Obviously cancer is far more complex than a single gene, but this is the notion we are working with.

JB:Let's take an example of an environmental factor that influences the non-coding RNAs, like a viral infection. I know you have done quite a bit of work in your history with HIV. If you have a viral infection, how does it impact expression of non-coding RNAs?

Research on HIV Infection and Non-Coding RNAs

KM:Well, HIV is a particularly interesting example. What happens is HIV can impact the microRNA pathway; that's well-known. How it impacts non-coding RNAs, which we have just now recently started to understand...I mean, there is so little work right now in the non-coding RNAs and there is not a lot of emphasis on it, so I can't go into the details of mechanistically how it impacts non-coding RNAs (and I'm talking about larger species of RNAs), but when we talk about microRNAs, which are smaller RNAs that are involved in regulating genes in a post-transcriptional manner, there is much more known about that, and HIV does impact that.

When HIV infects a cell, it uses the proteins that are involved in the microRNA pathway and pulls those proteins away from the endogenous microRNA pathway. It uses them for its own production, and by doing that, it causes a cell to become less regulated, less controlled, because it no longer has the proteins it needs for the microRNA pathway to regulate all of the endogenous genes so genes start to get out of whack. But the virus is coming in there, it uses that protein and it makes more of itself. The virus doesn't initially care; it wants to make a lot of virus, right, and produce more virus? But HIV will actually go into

a latent state-it will become hidden-if the cell survives and it makes enough virus, it goes into a latent state, and that's why we have this chronic infection that never goes away. We suspect, and we have some evidence that there are non-coding RNAs that HIV makes, itself, to regulate its viral latency, but that's not been published yet.

JB:So if we were to then ask a kind of a teleological question...I know this is an unfair question, but I'm sure you have a speculation. In the development of our biology, what advantage is there to have these non-coding RNAs in terms of their ability to regulate? Because clearly it appears like one might say, "Well, this sounds like a random, kinetic, molecular modulation of gene expression because you've got all this sea of small RNAs floating around, hitting on specific portions of the genome; it sounds like it would be a fire drill."

KM:Exactly, and we don't know where the non-coding RNAs come from. But the notion would be that...we're just going to talk about it on a cell level, but obviously the human organism, the human body, is an amalgamation of cells, but on a cellular level, the notion is a cell gets placed into an environment, it has certain interactions with that environment, and it causes a certain gene regulation. If that gene regulation is selected for (is positive), the cell out-proliferates the other cells. That cell would putatively have a particular non-coding RNA profile that is going downstream and regulating genes in a differential manner, and if that is selected for, those non-coding RNAs would then lead to epigenetic modifications that become sustained and kept, and that cell outcompetes the other cells and becomes the dominant cell. The thought is that the non-coding RNAs are involved in differentially regulating gene expression to allow for sickness to occur. There is absolutely no evidence on this; this is all speculation at this point.

JB:So now you have touched on something that has a lot of, kind of, sense within our listening group about what they call the folate cycle in intermediary metabolism, because we've discussed methylation and silencing and promoter regions of genes by the cytosine methylation patterns, and I think there is this sense that nutrients that support methylation, which would be folate, B6, B12, and betaine, for instance, can help to maintain proper methylation patterns, but we also know-as you've already alluded to-that there are promoter regions of genes that are hypermethylated, and does that occur as a consequence of, say, a person consuming too much folate, B12, or B6? It sounds to me, from the way you are describing this, that the regulation of methylation patterning is more complex, obviously, than just the available of s-adenosylmethionine. It is regulated, in part, by these small interfering RNA's communication that maybe direct methylation patterns. Am I moving in the right direction?

Small RNAs and Directed DNA Methylation
KM:Yes, sort of. If we design the small RNAs in our lab and put them into cells, we can see directed DNA methylation at the site we are targeting. It is not found upstream of that site, it is found downstream in the orientation of transcription. So it is as if the RNAs guide a complex that contains DNA methyltransferase 3A and Argonaute-1 and histone deacetylase-1-those three we know are involved. It is as if small RNAs guide that complex to that target site and it moves downstream in the orientation of transcription, causing changes in the nucleosomes (epigenetic changes-histone methylation, and eventually DNA methylation). We see DNA methylation-it occurs-and certain promoters are more susceptible to DNA methylation and they become silenced when they are methylated. But no one has really understood what is guiding the methylation to this site. Obviously the folate cycle is involved in methylation, and there are certain nutrients that are involved in maintaining the ability for methylation to occur, but the guiding mechanism, or the endogenous infector molecule that is telling the cell, "This gene

needs to have methylation at this loci," has been sort of missing. We suspect, with the evidence that is mounting in our lab as well as in others, that non-coding RNAs do this, and when they become dysregulated, or differentially regulated, they can lead to robust DNA methylation that can lead to long-term silencing, and that is sort of what you see in cancers, you know, with tumor suppressor genes that are turned off.

JB:So then that leads, obviously to a companion question, and, again, I'm coming back to our field in which the clinicians may be everyday asking, "Gee, am I influencing, somehow, the regulatory pathways through the way that this person is living their life that ultimately expresses itself through the phenotype?" We've heard a big story that comes out of David Sinclair's lab at Harvard: the resveratrol connection to histone deacetylation and the sirtuin gene family. It would suggest that there are maybe phytochemicals, or let's call it small molecules in the diet, that might actually have some influence on these regulatory pathways, but they way I'm interpreting what you are saying is that the impact of some of these might be much more complex in terms of their regulatory effects, mechanistically, going through small RNA-directed pathways. Is that a possibility?

KM:Yes, certainly there are small molecules and compounds found in our diet that can affect histone methylation and DNA methylation. The notion would be that the non-coding RNAs are guiding epigenetic changes to particular gene loci, but then it's the diet that is there to revert those changes, and that's functionally beneficial to the cell, then what you would expect is that that gene would be selected to be turned on, even in the presence of the non-coding RNAs. We don't know where the non-coding RNAs are coming from, but I would suspect that if a particular gene has been selected to be turned on based on the diet and it being beneficial for the organism, then I would suspect that you would find those non-coding RNAs where they are emanating from, their promoter, if you will, would probably become epigenetically modified. I think it's a balancing act in transcription, where you have the sense and antisense transcripts. The sense becomes and makes the mRNA, which makes the protein; the antisense is the non-coding RNA, and that's the 25{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} of the transcripts I was talking about, and those antisense non-coding RNAs are functionally involved in regulating the sense transcription. So I think there is a balancing act between the two, and I think diet and environment is the regulatory mechanism, but that hasn't been shown yet. We are headed that way. I mean, this is getting real complex real quick.

JB:Thank you. You are taking us exactly in the right direction that I think many of our listeners want to go. It seems like these discoveries you and your co-investigators are making can be applied maybe in two different ways, one of which is (you've already alluded to), we could conceivably see the construction of synthetic derivatives of these antisense RNAs that could be used therapeutically to interfere in specific ways with gene expression that would be favorable to a disease state, so that would be a pharmacological application, and there are many small biotechs that are kind of taking that concept up to develop these new molecules through the I & D process. And then the second would be to look at how the environment (diet, lifestyle, chemicals, xenobiotics, and things of that nature influence the regulatory pathways through these small RNAs and ultimately find out how to personalize nutrition through the appropriate kind of screening device. Do you see the latter, in the future, is going to be a possibility?

Potential Applications of Small RNA Research
KM:I think you hit the nail on the head, right there. There are two potential areas, here. I will start off

with the synthetic potential, here, in terms of making compounds that can specifically target gene expression. What is important about the most recent PLoS Genetics paper that we published is that it shows that if you find the non-coding RNA that is involved in regulating a particular gene and involved in turning that gene down, and you target that non-coding RNA in a post-transcriptional manner, you can inhibit the inhibitor (silence the suppressor, if you will), and that causes a gene to be turned up. Conversely, if you target a gene's promoter in the transcriptional manner, or you target the gene's mRNA in a post-transcriptional manner (most of the biotech companies are actively targeting post-transcriptional silencing), that's a transient approach versus transcriptional, which is more permanent because it involves epigenetics. But either way, if you target a gene's promoter, or the mRNA, you can turn the gene down.

So what is really remarkable about this RNA-based technology (this antisense RNA technology is what it really is--small interfering RNAs, single strand and antisense RNAs), is they can both drive transcriptional silencing. What is really fascinating about this is that we can specifically turn up or down a gene. You basically pick it: what do you want to hit? That's the notion. We can do that on a cellular level. Obviously delivering it inside the human body to the target regions...that's where the trick is. How are we going to get these small molecules into the cells that need it and not the cells that don't need it?

What is fascinating about the ability to be able to specifically turn up or down a gene's expression is that it allows (in my mind-I can see the light at the end of the tunnel) for the first time, where we can take and make personalized approaches. We can say, "Right. Patient A comes in. Patient A has a certain pancreatic cancer, let's say. They have a certain genetic profile (genetic expression mark, if you will) in this particular tumor area in the pancreas. These genes are up and these genes are downregulated and they are all known to be involved in pancreatic cancer." We can go in and design molecules that would cause to turn down those genes that are overexpressed and turn up the genes that are underexpressed. We have the potential ability to do that.

The question would be, how would you deliver it to the area where you need it, specifically, and not hit all of the other cells? That is the same problem we have right now with current chemotherapy. Now, you could do the same sort of approach dietarily. If you understood how diet works, and the small molecules that are being produced by a certain diet, and how that affects gene expression, you could tailor a diet to fit with what a person is needing in terms of gene expression (if you understood the diet on a gene expression level). Each person is different, right? There are different diets, there are different genetics, and there are different environments that they are dealing with, but certainly I see medicine moving (at least, in the next 20 years in developed countries) into a personalized sort of arena, where we can draw somebody's blood and a week later tell them, "Here's what the situation is. Here's what we can to amalgamate the situation (or ameliorate it, if you will)."

JB: So when we look at this future application that will come out of these discoveries, it sounds to me like we are into a whole, really different field of investigation that takes us beyond Mendelian genetics, takes us beyond the deterministic model that really medicine grew up around Mendelian kind of cross-overs and dominant and recessive characteristics, and it moves us in to a field of genomic expression plasticity, which is pretty empowering for a different kind of medicine than that which just treats rigidly defined disease that appeared as if it was a legacy of bad genes.

KM: Right. You are exactly right there. I mean, the Mendelian genetics still apply. We're not saying anything of what science has done over the past 200 years is not appropriate; it all fits in. And proteins

are still involved in gene transcription. We are just saying, "Hey, there's another layer of complexity that is going on, and we're just now getting to understand that because we have the technology that allows for us to go there and to figure these sorts of things out." I think the dogma in science is ridiculous because many folks are still stuck on this, "No, proteins control gene transcription." Well, RNA does, too. There are other factors playing out and it is shortsighted to think it is so simple. But maybe that is the way we want to be because it makes it easier to think about it if it is simple in sort of a Mendelian genetic approach. But yes, you are absolutely correct. There is a plasticity that is going on, and that's different for every individual. Diet affects that, environment affects that, and the body and cells respond according to a particular situation.

JB: Do we know if the telomeres have any influence on compaction of DNA such as to make the nucleosome more responsive to small RNAs?

KM: Telomere and telomere shortening...I don't know the exact specifics of that, but that probably does have a role to play in nucleosome structure, but see, the telomeres are at the end of the chromosomes, so I would imagine there might be accession points there for RNA polymerase and other transcription factors that may bind in there and start moving down the chromosome, so it might have to keep it relatively open. We haven't gone and tried to target telomeres to see if we could modulate their local nucleosomal structure. It was something we thought about doing with another group, but you know, resources are limited right now and we have to focus on what we can do.

Small RNAs and Telomerase Activity

JB: The reason I kind of went off on that sidebar is if you look at the Hayflick's number in replicating cells and you assume that that has something to do with shortening of the telomeres and the telomerase activity is important for maintaining the integrity of the telomeres, and now we look at this recent paper that appeared in Lancet-I'm not sure if you saw it-that came out of the UCSF Medical School group with Dean Ornish being one of the investigators...16 In this paper they showed that by intervening in people who had prostate cancer with a lifestyle intervention (this was a vegetarian diet, stress management, and exercise), they actually got enhanced telomerase activity, suggesting that they were possibly changing the integrity of chromatin, increasing genomic stability through increased telomerase activity, and that actually correlated with lowered LDL levels. It starts raising at least a question as to whether this would influence, then, expression activators (or modulators) of expression like small RNAs?

KM: I don't doubt it at all that doing those sort of lifestyle changes would affect your body, each cell, on a genome-wide scale. I would imagine it is a fantastic study that could be done to just take some patients and, you know, have the before and after and take the cells and do chip-on-chip sort of assays and look at where the nucleosomes are positioned. I mean, I can see doing an experiment in this manner. I would be willing to bet that there are significant changes that are occurring; it doesn't phase me at all. Now, how it is working...I think it is a complex scenario. I'm sure RNAs are involved. I'm sure non-coding RNAs are involved to some extent in the changing of the nucleosome positioning.

JB: One of the important takeaways that I have from what you have said-and by the way, you've said it brilliantly, at a level that we as non-specialists in this area can understand-is the question that has been floating around, and that is, if something is deficient and you increase the intake of the precursors to modulate that deficiency (now I'm thinking of something going back again to methylation of the genome with folate-related nutrients), it doesn't necessarily mean excess of those same substances would cause a

hyperfunction because there are so many regulatory steps in what does and does not get, in this case, methylated, other than just the availability of the methylating agent s-adenosylmethionine? So if you don't have the methylating agent there at all, then it is hard, no matter what the regulatory mechanisms are, to methylate. But if you do have the methylating agent there in adequate levels, there are many other factors that will control and regulate regions that might be hypermethylated or hypomethylated. Am I saying that correctly?

KM: Right.

JB: So when that comes back, then, to your "a-ha" for us-25{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} of the genome related to these non-coding RNAs-it would suggest this is a fairly important regulatory conserved feature of mammalian physiology.

KM: That's the notion. As I said, there are very few of us working in this area. I suspect more and more folks will start working on it as it becomes more realized. Getting into the methylating agent and having...it's a substrate versus enzyme, right? And if you have a lot of substrate there and no enzymes, that could also impact the ability to methylate, if you will. I suspect there is a balancing act that is required. As is always the case, the middle road is probably the best one to take for a healthy individual and a balanced sort of expression profile that allows itself to deal with environmental stresses and strains and to evolve accordingly to the selective pressures placed on it.

JB: Let me ask one last question about these siRNAs (the small interfering RNAs, these antisense RNAs). If you were to do some speculation here and kind of look forward five to ten years, how do you see this translating into a clinic? Do you think we are actually going to see some leverage of these discoveries at a translational science level?

Looking Ahead to Clinical Applications of siRNA Research

KM: Yes. Like I said, I've been doing science for several years and I used to think, "Oh, we wrote another paper, published it, no one will read it." I never saw any sort of translational aspect of what we did. It is just now that I'm saying, "Wow, this can really go." In my lifetime I can see the work I'm doing, and several thousand other people are doing, actually becoming developed into a drug, into compounds that are useful. As I said, delivery is an issue. But delivery is always an issue in every drug you develop. But I do see that we are headed that way where we can design these compounds-small interfering RNAs, small antisense RNAs, and small non-coding RNAs-we can develop this sort of technique to turn off or turn on a gene. It just is a matter of what genes need to be turned off and what genes need to be turned on. And we can figure out what genes need to be turned off and turned on based on our genome-wide screening capabilities that we have, and then once we have that, it is a matter of finding the small RNAs that will work to turn off or to turn on the gene, depending on what you need to do. Once you have those, you have those banked in a library. Data is data, and you make the molecule. And then the last question is, how do you get it in to the cell? If it is pancreatic cancer and if they are doing surgery, you can get it into the local environment that way. That has always been sort of the issue (the delivery), but I really do believe that we will see this in the next 10 to 20 years. I'm optimistic, though.

JB: I think your optimism is well-framed, and I think this concept that some of these are heritable and how that will be seen as another way of modulating the health of future generations-these are very

powerful concepts that you are discovering. Congratulations, I saw that for 2009 you have been awarded an Astor Fellow at Oxford. That is certainly another indication that your work is being seen as meritorious and having value. It sounds like your future to make real contributions in this area is very rich.

KM: Well, let's hope so. I mean, I take it day by day. Yes, the Oxford Fellowship is very nice. There are some great folks over there and it will be a nice time to go over there and visit and teach about some RNA-based regulatory mechanisms. We'll see. As the Chinese proverb says, the future is difficult to predict.

JB: We wish you the very best and we thank you so much for spending some time with us. This has made available to a large group of clinicians a topic that otherwise they might have been pretty intimidated about, so thank you so much.

KM: Thank you, Jeff.

We thank Dr. Morris very much for his extraordinary job in making a very complicated topic understandable. As you know, I was talking (as an example) of environmental agents that can modulate epigenetic and genetic signaling in an earlier section of this issue, the vitamin A and vitamin D connection. I was talking about the necessity for providing adequate levels of vitamin D to meet the needs of the individual such that the blood level is somewhere in the range of about 40 – 50 nanograms per milliliter of the 25-hydroxyvitamin D, kind of a standard test that we should be doing to evaluate vitamin D status in our patients. We also recognize that excessive levels of either vitamin A or D can tip us on the other side of the curve and produce adverse effects. We can't assume that just because it is a nutrient that if a little is good more is better. We need to be in that zone of proper optimal function, and for vitamin D that is somewhere around, apparently, the 40 to 50 nanograms per milliliter level of the 25-hydroyvitamin D in the serum.

In Closing: Articles on Coenzyme Q10 Supplementation and Statin Mypopathy

One of the other interesting associations between supplementation of nutrients and function that is emerging is the coenzyme Q10 association with statins. Recently, there was an article in the *Harvard Health Letter* (in their September 2008 issue) about the coenzyme Q10/ubiquinone relationship to mitochondrial function, the powerhouse of the cell.[17] It is recognized that taking a statin lowers coenzyme Q10 as a consequence of interruption of its biosynthesis. In this article, the author says taking a supplement increases blood levels of coenzyme Q10 if it is a bioavailable form of coenzyme Q10, but the effect inside muscles is inconsistent. Whereas one study showed an increase of co-enzyme Q10 in muscles after supplementation, another showed a decrease, indicating that the form of Q10 may be very important in establishing its efficacy. More to the point, they go on to say that only two trials of co-enzyme Q10 for statin-induced muscle problems have been published up to this point [in 2008]. Since then, we have seen a number of other studies that have been published that once again reaffirm the value of supplementation of coenzyme Q10 when people are taking statins as a conditionally essential nutrient, meaning that it is essential on the condition that they were interrupting the synthesis in their bodies of co-Q10 due to the taking of statins, and that this seems to correlate with lowered Q10 levels and increasing risk to myopathy, one of the major side effect of statin supplementation.

Recent studies that have been looking at this in greater detail include a very interesting paper that was

published in 2008 in *Current Opinions in Rheumatology* titled "Genetic Predisposition to Statin Myopathy."[18] In this particular paper the authors ask, why do some people appear to be more susceptible to statin myopathy than others? And they did find a genetic analysis for variants and disease-causing mutations relevant to statin myopathy seem to provide a better understanding that there are some people who are more likely to get myopathy from statins as a consequence of the effect it has on mitochondrial energy metabolism, and that they are these kind of yellow canaries who first show apparent functional Co-Q10 insufficiency.

The concept is finding the right patients, giving the right dose, and making sure it is a bioavailable form. In a more recent paper, researchers looked at the effect of ubiquinone/co-enzyme Q10 on myopathy in statin users.[19] This was published in *Current Opinions in Lipidology* in 2008. Here was kind of a meta-analysis of looking at studies that have been published on supplementation of coenzyme Q10 showing that bioavailable forms do increase blood levels, tissue levels can vary from patient to patient based upon their individual absorption and transport properties, although the overall evidence from the literature does not support coenzyme Q10 supplementation in statin-induced myopathy, says the paper, there are those notable exceptions where people seem to have had a very remarkable response to co-Q10 given at several hundred milligrams a day of a bioavailable form in the reduction of their myopathy.

And then lastly, the most recent paper is one titled, "Coenzyme Q10: Is There a Clinical Role and a Case for Measurement?"[20] In this particular paper in *Clinical Biochemical Reviews*, the investigators go on to say that coenzyme Q10 is an essential co-factor in mitochondrial electron transport, and that measuring blood levels of Co-Q10 might help us to understand those patients with myopathy that would be potentially candidates for Co-Q10 supplementation, and then supplementing them in a dose-response relationship, titrating their need with a bioavailable form of Co-Q10 to bring their plasma level up, their tissue levels up, and to monitor their symptoms, might the best way proceeding on therapies. So they come to the conclusion that there is individual variation in Co-Q10 absorption and utilization, but correlating the clinical symptoms with the plasma Co-Q-10 levels might be very helpful in defining those patients more likely to respond to Co-Q10 supplementation.

In closure, what have we said this issue? We have said that there is genetic variability, there are environmental modifiers, and that one size doesn't fit all. When we are doing a supplementation program, it should be more of a therapeutic intervention, individualized to the person, but we need to start with a good diet that is rich in an array of phytochemicals and delivers low glycemic load and has an effect on normalizing these hormones that are involved with inflammation signaling. I think with all of this packaged together, we can now start to explain some of the molecular interactions at the cellular level that give rise to gene expression patterns and emerge the first steps towards, really, the definition of a personalized functional medicine. Thanks for being with us. See you next month.

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