

October 2006 Issue | Richard Deth, PhD Northeastern University

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Welcome to *Functional Medicine Update* for October 2006. Functional medicine often deals with the underlying mechanisms that inter-connect different diagnostic codes to try to provide an explanation of common cause for what later is seen as differing pathologies. It is that detective-type of story that really highlights (and gives a specific personality to) functional medicine. It is like putting together a puzzle: taking different pieces of information from clinical presentation, history, antecedents (such as in the genes), and environmental factors (often called "triggers") that then lead to the modification of mediators, which results in the signs and symptoms of a disease.

The emerging concepts that underpin functional medicine are codified very nicely in the *Textbook of Functional Medicine*. True functional medicine, however, requires some pretty detailed detective finding and some searching by the clinician. Some people are much more suited to this than others, as can often be seen in how a person approaches his or her life. For individuals who like a detective story and like putting together different bits of information to create an outcome of understanding, functional medicine provides a formalism to achieve that objective.

One of the most interesting examples related to how this model can be applied is in the area of neurosciences and neurological conditions (what is sometimes termed as "behavioral neurology," the interface between psychiatry and neurology). There are a variety of diagnostic codes-things like attention disorders and hyperactivity disorders; various forms of schizophreniform disorders; cognitive deficit disorders; and also autistic spectrum disorders. All of these have very complex etiologies and interrelationships of environment, genes, and basic neurochemistry.

In this issue of *Functional Medicine Update*, we are going to take a new look at a story that we have heard about before in *Functional Medicine Update* over the years: the homocysteine story. You may recall that we interviewed Kilmer McCully-the father of the theory connecting homocysteine to cardiovascular disease-talk about his 1969 discovery connecting elevated homocysteine and vascular disorders. Since then, there have been a lot of controversial papers on both sides of the table on this-some saying that vitamin supplements with folic acid and B12 will lower the risk to homocysteine-induced cardiovascular injuries; some saying the say the opposite (most recently, a paper in *The New England Journal of Medicine* titled "Homocysteine Lowering with Folic Acid and B Vitamins in Vascular Disease" in which it was concluded that there is no benefit from B vitamin supplementation¹). In this issue of *Functional Medicine Update*, we will be discussing the concept of behavior neurology. We will start with our interview with a clinician of the month who I think has a tremendous concept to share with us, Dr. Richard Deth.

INTERVIEW TRANSCRIPT

Clinician/Researcher of the Month
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JB: Once again we are at that part of Functional Medicine Update when we have the privilege of speaking to someone who is doing something that I think represents where the field of functional medicine is going—the trajectory of change. Our interview this month is with Dr. Richard Carlton Deth, who is a professor of pharmacology at Northeastern University in Boston, Massachusetts. He has been there for a number of years as a full professor; he is also a past chairman of the department of pharmacology. He has been involved with pharmaceutical sciences now for more than 25 years. His research publications are many, and you are going to hear much more from him about the areas that he has focused on for the last 10 years or so. These have to do with molecular mechanisms that underlie autistic spectrum disorder and biobehavioral changes (cognition changes).

I think Dr. Deth brings a unique and incredibly insightful perspective to these topics, which translates to real clinical benefit. We will even have the chance to talk with him in this interview about his congressional testimony. There are many parts to the series of discoveries that Dr. Deth has made that I think you will find fascinating. Dr. Deth, welcome to Functional Medicine Update.

For our listeners, I think that the way we might start into this is to have you tell us a little bit about the D4 dopamine receptor activity and its connection to cognition; I know that is going to lead us down a path to discussing some of the discoveries you have made over the past several years.

An Introduction to the D4 Dopamine Receptor

RD: Thank you, Jeff. It is my pleasure to be here; I appreciate the invitation. Let me try to introduce the D4 dopamine receptor. In doing so, you'll recognize that the neurotransmitter dopamine, of course, has a family of receptors—that it exerts effect times 5, to be exact. The D4 dopamine receptor, which is our focus here, is the only one that carries out this process that we discovered, in which it is able to transfer methyl groups (that it receives from the folate pathway). It transfers them one at a time, but very rapidly, and places them on the phospholipids that surround the receptor, in the membrane of nerve cells. The D4 receptor is typical of G protein-coupled receptors (a big family), but it is the only one that can do this job because it is the only one that has methionine residue. That is the critical chemical location necessary in order to be able to carry out this process.

Just by way of personal recollection, how we got started on this was because we were doing molecular modeling studies using the technology of computer graphics and studying these G protein coupled receptors, and then we particularly focused on the location that happens to be where this methionine is. We were studying other receptors, and the process we were interested in had to do with why they had spontaneous activity, and it turns out that this methionine location is involved in that; it regulates the spontaneous activity of these receptors.

The D4 Dopamine Receptor and Cognition

The D4 dopamine receptor is very interesting in humans and primates, in particular. After we noticed this methionine, which is-again-a unique feature, we started to look at the general literature of the D4 receptor. In about 1995, I guess it was, there was a study (the first one) linking it to ADHD, obviously a problem related to the role of dopamine and attention that involved the D4 receptor. The link that was brought out at that time was that a particular feature called a "repeat" (a structural feature on the cytoplasmic side of this receptor that was variable in humans as a one-to-the-other) was found to be a risk factor, if you will, for ADHD. This broke down to if you had 7 of these repeats, then your risk of ADHD was estimated at 3- to 5-fold higher than if you didn't have the 7-repeat form. So this was intriguing and it gave the first clue to us that that receptor might have a unique role to play in attention since variations in its structure apparently were related to variations in attention.

The number of repeats is one feature of the D4 receptor, but at the same time there are 35 different sequences (or probably more by now) that are possible for each of those repeats. So there is a fantastic variety from human to human in terms of the D4 receptors makeup and its structure. The function of these repeats is to hold other molecules-other proteins and channels and transporters-to the receptor; it's a binding site where they can be held by the D4 receptor. We think that this feature goes hand-in-hand with the methylation of the membrane by the receptor (as dopamine stimulates it) because these other proteins that are bound to it become targets-they become things in the neighborhood (or the microenvironment) that can be modulated or affected by the methylation of the membrane that we discovered.

All of these things, together, make for a very interesting signaling complex involving dopamine and attention. Really, that is how we got started and it sort of led us to all the other features, like autism.

JB: That is a really interesting background. For the sake of our listeners-often we have people with a variety of different strengths in the area of biochemistry and molecular genetics-I just want to summarize it. What you are referring to, obviously, are these receptors that are protein in nature. They are coded for by our genes, so we have unique polymorphisms that are possible, and in this particular receptor there are possibilities for a repeat of certain amino acid sequences that then change the structure function of that receptor and can alter, as you said, its ability to transfer methyl groups to the phospholipid core of the membrane, which then, in turn, changes receptivity of the membrane and its fluidity. It sounds like a very dynamic dance that has a lot of genetic underpinning, and-you are going to tell us, I know-also some environmental factors that can influence this.

RD: You did a very good job of summarizing that.

JB: Thank you. One of the things that you said in your papers, which I found fascinating-I never had recognized this-is that all mammals have this DR4 (dopamine receptor 4), but there is a very interesting relationship between cognition in the animal and this repeat frequency, and so you can actually kind of do a map of cognition or attention in the animal's own DR4 genetics. Could you tell us a little about that?

Mapping Cognition

RD: Well, this is an area that is still sort of being worked out. I mentioned that these particular repeats were restricted to primates, that is, the nature of them turns out to be 16 amino acids involved in each of the repeats, 48 nucleic acid bases at the level of the gene, and that's what primates have. You have to have at least 2 of these repeats which aren't present in other species to be a primate, if you want to think of it

that way. But, there are similar kinds of repeats, not exactly the same as the human primate type, but there are similar signs that other species also have modifications (or repeats) within their D4 receptor structure. These other species include dogs, whales, and animals to which we tend to attribute the ability to think in certain intelligent ways. While that is really an area that is early in its clarification, it might mean (and I think it does, at least) that whatever process this dopamine receptor facilitates might be very important for the function of the mind as a whole. So, we will have to wait and see, but we think that (and we have proposed) it is involved in synchronizing parts of the brain to be able to work together, more or less, as a whole (sort of integrating parts of the brain).

JB: That leads us, then, into a question as to what is the proposed relationship between DR4 activity and polymorphisms and ultimately conditions like ADHD or autism?

The Relationship between DR4 Activity and ADHD and Autism

RD: Well, I mentioned that the 7-repeat form was linked to ADHD and we were measuring this methylation activity, also, in different receptors with different repeats, and we found that the 7-repeat form was weak in carrying out methylation in response to dopamine. This caused us to think that the weakness of methylation might end up being a weakness in attention, and if that was true, it could sort of join up with that genetic risk-factor idea.

Reasonably, we know that there are really two activities of dopamine that help support this overall process. One has to do with changing the shape or the confirmation of the receptor. The second has to do with stimulating the supply of methyl groups to the receptor. It looks like the 7-repeat stimulates the supply of the methyl groups to the enzyme methionine synthase very well. Some factor associated with the receptor's shape, or other features of the physical properties of the receptor are sort of less favorable, and so there is a balance of things, but it appears as though the 7-repeat form is associated with a risk of ADHD.

What I think goes on (just to be more precise about it) is that the process by which the dopamine D4 receptor normally makes its own methyl supply better (or adequate) involves the activation of the enzyme methionine synthase. This is the enzyme-the B12 and folate-dependent enzyme-that brings methyl groups to the receptor and, therefore, has to get a new methyl group every time a previous one is donated. It looks like D4 receptors stimulate that enzyme, and they do it by helping the enzyme to create methyl B12, or methylcobalamin, in a glutathione-dependent manner. This is an area of work that really gets started from an observation made by Dr. Jim Neubrandner, which you may have heard about (many people now have). He observed that methyl B12 (or methylcobalamin) was having unique therapeutic effects in a significant number of autistic kids, and so it was a challenge to us to relate that to our D4 receptor work.

JB: What you are describing is absolutely an example of functional medicine. You are crossing what were considered separate channels in traditional science and medicine. We know Dr. Neubrandner very well-he has been a functional medicine supporter for many years-and to move from the clinical world to the genetics world, back to the clinical-biochemical world, and then to the neurological sciences world-and to do it with such ease-is, to me, the embodiment of what functional medicine is all about. I want to applaud your willingness to do this because I know that often in the field of scientific research we are not supported for doing that; we are often criticized because we've cut across somebody else's domain.

RD: You're right. With the way both medicine and research are practiced and explored, there are these

boundaries, which are not fixed, but are quite real, nonetheless. In our case, it is pieces of molecular puzzles. In some cases, puzzles of the disease, such as autism or ADHD, but in other cases, a puzzle of how nature accomplishes something. Those boundaries disappear, really, when you approach problems from a practical standpoint of trying to figure out the puzzles. More or less, we have gone step by step and discovered, and actually taken on, new areas of understanding and learning-whatever is necessary for the next challenge, or the next step in the process that has presented itself.

JB: Let's get back to your story; it is very exciting. We have now said that the DR4 receptor biochemistry has some genetic polymorphisms. That means that there are things which can vary its ability to transfer methyl groups up to the membrane, and that methionine synthase is involved through the methylation transfer reactions. This also means an interrelationship to the folate cycle and the B12 connection exists, which leads to the questions: What are the natural methionine synthase agonists that generally turn on this methylation pathway? And how do these vary from individual to individual?

Methionine Synthase and Homocysteine

RD: The methionine synthase is an enzyme, rather than an agonist like turning on a neurotransmitter receptor, we are thinking here of an enzyme, which carries out a reaction and which needs certain cofactors and conditions to function effectively. Of course, its reaction is to take homocysteine (formed through the methionine cycle, as it's called) and convert the homocysteine back to methionine by attaching a methyl group from methyl folate (tetrahydrofolate).

It does that by temporarily taking the folate-derived methyl group and attaching it to cobalamin (or to, actually, the cobalt atom in the B12 or cobalamin), and then the methylcobalamin sits in the active side of the enzyme and waits for the homocysteine to be brought close in. When homocysteine is close enough, the methyl group is transferred to the homocysteine, and becomes methionine. With all of that having been done, the cobalt of the B12 is now bare again; it just needs to be refilled from the folate to carry out that cycle again. In the case of the D4 receptor, the ability to carry out this cycle is directed toward the methionine that is attached to the receptor, the one that is passing the methyl group along to the membrane phospholipids.

What we proposed, and what we have now certainly very clearly shown, is that the anti-methionine synthase had two substrates. On the one hand it has the homocysteine, which is what it is certainly best known for. Elevations of homocysteine in various diseases-cardiovascular, atherosclerosis-related diseases, but also now in both schizophrenia and Alzheimer's-probably also reflect problems with methionine synthase activity, but in addition to the homocysteine itself (as a substrate), we have the D4 receptor in its homocysteine state. The efficiency of this dopamine-stimulated phospholipid methylation depends critically on this enzyme. Anything that alters its activity-or its ability to deliver methyl groups to the receptor-is going to result in a loss of something like attention, or whatever role the D4 receptor plays in attention.

JB: Is homocysteine elevation a plasma biomarker for this condition, or are they not really closely correlated?

Elevated Homocysteine as a Biomarker

RD: To my knowledge, there has been no description of an elevated homocysteine level in ADHD, for

example, and certainly it is not elevated in autism, but I was referring to some conditions in which it is elevated. It is elevated, as I said, in schizophrenia, especially in first-episode males; it is very clear and one almost could consider it a biomarker there. And then in Alzheimer's it is very commonly measured and has certainly been widely confirmed. But it is not specific for those conditions, of course. Elevations of homocysteine are associated with different conditions involving different organ systems and different tissue types because it is such a general metabolic process in all cells.

JB: Do anti-folate medications have any adverse effects, clinically, on autism or attention deficit disorders?

RD: That's an interesting question and I'm thinking of methotrexate, widely used for leukemia. I'm well aware of neurological syndromes precipitated by methotrexate-but, again, it is in vulnerable individuals. Methotrexate, in and of itself in the broad population, does not typically produce neurological problems. But, if there is a background of vulnerability (typically genetic) that impinges also on these same pathways, and with the addition of methotrexate (for example), the lack of supply of methylfolate is brought about, then in conjunction with those other risk factors, a syndrome can develop. This is an interesting parallel to autism, more generally, where we think that people (kids) would have done fine in their lives and would not have suffered autism if it weren't for some precipitating factor, not methotrexate, commonly, but certainly other environmental factors-heavy metals certainly being near the top of that list.

JB: I want to move to that and take a little sidebar summary. What we have said so far is that those things that support proper methyl transfer from the DR4, which would be things like the folate cycle nutrients (B12, 5-methyltetrahydrofolate, B6, betaine [as a methyl transfer component]) seem to be helpful. Then we go to the other side of what are things that might actually inhibit methionine synthase and cause problems (in the susceptible individual) in the transfer of methyl groups to the phospholipids. That leads us to one of your great additional discoveries around the heavy metal connection, so I would like to ask you about the lead and mercury part of the story, which I know has been a major advance that you've brought to our attention.

The D4 Receptor and Heavy Metals

RD: As we pursued the factors that are important for the D4 receptor (the methionine synthase activity), we recognized that there is a role here for glutathione and for the redox state of cells. In particular, in neuronal cells and neurons and in the brain and in the cortex (just to be quite specific about that), the methionine synthase exhibits a specific and an absolute requirement for methyl B12 (or methylcobalamin), and the methyl B12 has to be synthesized by a glutathione-dependent pathway. Even if we consider the pharmaceutical forms of B12 like cyanocobalamin, that must be converted first to glutathionylcobalamin, as an intermediate, and then the glutathionylcobalamin gets converted to the methyl B12, and the methyl B12 is essential for methionine synthase activity in the cortex and in the neuronal tissues. It is not essential in the liver and in other organs because the enzyme is configured differently in those other organs. In the brain and neuronal tissues, the ability of methionine synthase to be reactivated when the cobalamin gets oxidized requires this exogenously supplied methyl B12 (or synthesized methyl B12).

The way that heavy metals come in is because heavy metals are a prime example of agents that cause a decrease in glutathione levels. We showed that mercury, lead, and thimerosal are all very potent inhibitors of methionine synthase, and that the inhibition occurs because these metals cause about 40 percent

decrease in glutathione levels in the cells. As a result, the methyl B12 availability is blocked, and accordingly methionine synthase activity decreases to an undetectably low level.

It was a very critical effect that the heavy metals had via their effects on the glutathione. Now that is important because, as Dr. Jill James' work has shown a second time in her recently released paper, autistic kids have decreases in glutathione of about 40 percent in their peripheral blood. That kind of a decrease certainly suggests that these kids don't have the capacity to make methyl B12, and therefore will have a deficit in methionine synthase activity and a deficit in the dopamine-stimulated methylation process.

JB: This is really fascinating. Again, we are looking at genetically unique individuals who, as a consequence of the way that their tandem repeats are seen in their DR4, may have differing relative susceptibilities to ineffective methylation of phospholipids and membrane transport phenomena, and that due to genetic uniquenesses certain individuals may be much more sensitive to these environmental agents-like heavy metals. And then you introduced the substance-which I want to come back to-"thimerosal," which I know has been a hugely controversial topic in medicine. Thimerosal is a preservative that is used in various types of products, particularly immunizations.

In one of your papers, or maybe it was in a couple of your papers, you had a very interesting bit of data that I was unfamiliar with that relates to the effect thimerosal has in cell culture. And then, you reported actual levels in the blood in children after they get an immunization. Could you tell us a little bit about that? That was news to me.

Thimerosal Data

RD: Yes. As we tested these environmental agents (and by that I mean lead and mercury, but also arsenic and cadmium had similar effects), we found that they were very potent. By that I mean the concentrations that could be described as "sub-nanomol," or really low concentrations (concentrations that were known to be associated with lower IQ). For example, in the case of lead, these concentrations detected in the blood have been very clearly linked to lead poisoning and neurological consequences. As we did response-type of experiments, we could gauge the potency of these agents. In the case of thimerosal, the relationship was such that at the concentration of 10 to the minus 9 (one nanomolar, as it is called scientifically) we had a good 50 percent inhibition of this process.

There have been papers published (by Pichichero, in particular-the one I am thinking of was in The Lancet) where they measured the levels of mercury that were produced in the bloodstream (the peripheral blood of children that were vaccinated). Their studies showed about a 10 nanomolar concentration in circulation as a result of vaccination, and that was even 10 days or so after the vaccination. Clearly, that concentration was enough in our system-the cultured cells, which is considered artificial. Still, we have to be kind of circumspect about these concentrations-the effects that we were seeing (the inhibitory effects on the enzyme methionine synthase) were occurring at physiologically relevant concentrations. That is important because mercury and ethylmercury and thimerosal can inhibit many (if not all) cellular processes at some concentration. I mean, if you have enough of it, you can kill anything; that is why it is used as a preservative. To implicate it in something that might be related to, let's say, mercury vaccines, you certainly had to show that it [the activity in question] is exquisitely sensitive to very low concentrations of the thimerosal, and in effect that is what we found.

Congressional Testimony on Thimerosal

JB: I was able to pull up a record of your testimony that you gave to the US House of Representatives

Committee on Government Reform and Rights and Wellness, and I think you also gave a similar testimony to the Committee on Appropriations, on the link between thimerosal and autism back in 2004. How did that go and how was it received?

RD: The political realm is certainly different from the scientific realm. The first testimony was basically Dan Burton's subcommittee, and of course he has been a champion of the anti-mercury cause (if you can say it that way) for a number of years and still is because of his grandson's autism and his conviction that it has something to do with mercury. Just to be frank, it was a subcommittee called for the purpose of bringing this to light and there were persons like myself offering testimony. I gave testimony and it became part of the congressional record and so you are able to access it. It serves that kind of a purpose, but it really wasn't involved in, let's say, a legislative process; it was like a hearing about something that a congressman was interested in.

The other hearing was actually a little more dynamic and a little more central. There was actually quite a bit of interest in that hearing. The basis for that appropriations hearing was to question the thimerosal in vaccines because of the flu vaccine (purchase of flu vaccines could be thimerosal-free vaccines or thimerosal-containing vaccines); this was really the basis for that. There were (if I can say it this way) some "big-shots" or important people besides myself on that committee. Julie Gerberding from the head of the CDC, for example, and Tony Fauci from the NIH were there talking about the value of vaccination, generally (they didn't necessarily advocate mercury).

In any case, the setting for that was a little more electric. There was coverage by CSPAN and there were reporters; it had the makings of a bit of a media event. What was interesting was that part way through that very hearing about thimerosal safety it was announced that there was a flu vaccine shortage. We all recall that there was this rush to get vaccines because of a problem in their manufacture, and there indeed was a flu vaccine shortage for a little while and then it went away within a few weeks and there wasn't a flu vaccine shortage. But it was just kind of interesting. There was a confluence of issues, and what the world heard about certainly had nothing to do with thimerosal and vaccination; it had to do with a shortage of flu vaccines and rushing out to get one would be a good idea.

JB: We have had the opportunity to speak with Dr. Herbert Needleman. Undoubtedly you know about his work with lead and IQ in children. I am just wondering if there is a connection between your discoveries that lead (as one of the heavy metals) could have an adverse effect on DR4 activity and its methylation and IQ?

DR4 Activity and Lead

RD: That is such a great question. I just heard the answer this last week. I just came back from a meeting in Little Rock, Arkansas where a speaker (unbeknownst to me) who was speaking about lead (Dr. Ken Dietrich, I believe, from Cincinnati) was presenting. He had shown that different concentrations of lead produced deficits in function (neurological deficits), and then he went back to look at the D4 receptor genotype in those individuals in the different lead concentration groups. What he showed (and presented here) was that if you had that 7-repeat form of the D4 receptor (the risk-introducing form), then indeed you had more severe effects of lead. He, therefore, was presenting this in the context of a gene-environment interaction-that is to say, that genetic feature made individuals more sensitive to the environmental presence of lead. It was very remarkable for me to be sitting in the audience, not knowing this investigator, and hearing that relationship. It really answered the question that you just asked in a

very clear way.

JB: That's fascinating. Obviously we have gotten to what for many of the listeners is probably the payoff question. That has to do with what are, in your estimation, the potential implications of this work, specifically for clinicians who are treating autism? Are there some "pearls" that we can take away? You have talked a little bit about the cobalamin, methylcobalamin, and the 5-methyltetrahydrofolate, the glutathione...can you guide clinicians as to what you feel is coming out of this as it relates to its implications?

The Clinical Applications of D4R Research

RD: Those things, in fact, are clearly coming out of this. In our most recent work, I just want to emphasize (it's very exciting) that the cortex requires methyl B12, and it requires glutathione to make it. Supportive treatments, which of course are very well known to functional medicine, that could raise glutathione levels (and by that I am referring largely to N-acetylcysteine, for example) would be very complementary to the administration of methyl B12, along with methyl folate or folinic acid (either one of which raises the levels of 5-methyltetrahydrofolate). That combination of three things would seem to be best targeted to support this process in the cortex (critical for that, as a matter of fact).

In addition, there are other modalities that we hear about that are interrelated to this. What this brings up is the importance of normalizing redox state in the cortex. To do that, those things that I mentioned certainly go in that direction, but so do a lot of the other things that are already being used (to name some: omega-3 fatty acids, by virtue of their nature, influence oxidative stress and reduce oxidative stress by a different pathway; and antioxidants of all different forms can be helpful here). To look ahead, hyperbaric oxygen is another thing that seems to be helpful in this regard. Lowering the levels of adenosine, which is a player that is also a part of this process, would be a useful therapeutic goal, and I believe that the HBOT (hyperbaric oxygen therapy) may be acting through that means when it produces benefit.

Then there is the interest in drugs that address inflammatory state (beyond NSAIDs and things like that). I'm thinking here of the so-called PPAR-gamma agonists, things like Actos and other glitazone drugs used commonly for type 2 diabetes, but also now being tested for use in Alzheimer's, for example. They also seem to be effective in autistic children. So there are a number of those things, some of which are clearer than others, and the methyl B12 issue now of concern is what dose should we be using and should we be delivering it through nasal, transdermal, or subcutaneous injection methods? Clinical trials and comparative studies are necessary to sort that out. So there is benefit, but then one needs to figure out how to optimize the benefit.

JB: I can't tell you how much we appreciate what you have done in this very short period of time. You have raised the level of our understanding an order or two of magnitude. Your work is pioneering and you communicated it so very well. It is a very complex topic, but you brought it to a level that we can understand.

Dr. Deth, I really want to thank you. I know this is a sacrifice of your time, but it will spread widely and I think many people will value from what you shared today. We wish you the best in your continued work and hope we can check back with you in the future.

RD: I look forward to that, Jeff, and thanks for having me on.

JB: It's my great pleasure. Thanks so much.

For many of you, you may have just had your mind expanded with the concepts that Dr. Deth was sharing with us concerning the methionine synthase connection to the DR4 genotype and how that relates to membrane methylation, how that connects to neurotransmission and to arousal and attention, and how that may then inter-relate with environmental factors, such as nutritional status and toxic environmental exposures to things like mercury or lead.

Dr. Roger Williams: The Relationship between Early Research and Current Findings

As I was listening to Dr. Deth and reading his papers, I flashed on a reflection of a conversation I had many years ago with one of the founding fathers of nutritional medicine. He is a paragon (as we have viewed his work in the development of this field) and that is Dr. R.J. Williams (Dr. Roger Williams from the University of Texas), the father of the concept we call "biochemical individuality."

Many years ago, when I was attending a seminar he was presenting, Dr. Williams said, "Nutrition is for real people. Statistical humans are of little interest." In light of the postgenomic era that we are now living in, and in the context of Dr. Deth's presentation, we are starting to look at this as a nutrigenomic question, aren't we? That is, the interrelationship with specific genotypes and environmental modifiers of function, both genetic and epigenetic.

The Concept of Genetotrophic Disease

It struck me that I should review and share with you what Dr. Williams was talking about in the 1940s. *Nineteen forties*-I want you to recall the context of this discovery. He was helping us to understand, early on, the nature of biochemical individuality and what he later termed "genetotrophic disease." This is a concept that seems as prescient and as current today (in light of Dr. Deth's comment) as it was ahead of its time in the 1940s.

The Discovery of Pantothenic Acid

For those of you not familiar with Dr. Williams (and I would be surprised if you listen to *Functional Medicine Update* and are unfamiliar with him), he was a prolific scientist, and he cultivated many extraordinary students and co-investigators. For example, Dr. Don Davis, a colleague of mine and a friend, was one of his postdoctoral (and later) research colleagues in the field, and he has continued to do extraordinary work. There were hundreds of young investigators coming through Dr Williams' laboratories. He was known as the father of pantothenic acid, having discovered it as one of the B-complex nutrients, and so he had earned his stripes as a very competent scientist and, in fact, at one time was the president of the American Chemical Society, one of the largest professional organizations for chemists. He was highly respected. If you want to read a classic, read the review of Dr. Williams' work on pantothenic acid, which was published in *Nutrition Reviews* in 1979. ³

Evaluating Nutritional Adequacy

Dr. Williams and his colleagues (including Dr. Davis) looked at all sorts of different ways of evaluating nutritional adequacy. What they went on to say is that there are ways of looking at nutrition adequacy from a pathologic perspective (like you would see with anemias or protein wasting, hypoalbuminemia, scurvy, beri beri, or pellagra), or you could use functional criteria for establishing nutritional adequacy. They published a paper back in the middle 1970s in the *American Journal of Clinical Nutrition* titled

"Potentially Useful Criteria for Judging Nutritional Adequacy."⁴ They looked at things like voluntary consumption of food, sleeping time after anesthesia, weight gain after surgery, healing time after surgery, hair growth after clipping, voluntary sugar consumption, and recovery time after cyanide poisoning. They varied the nutritional status of the animals and then they looked at those functional criteria and showed that there was very marked difference in functional response to each of those parameters that would be early warning indications of undernutrition, well before you saw frank malnutrition (the pathological signs of scurvy, beri beri, pellagra, or whatever it might be).

They then asked the question: how can the climate of medical education change to incorporate some of these concepts into teaching so that doctors are more attuned to these early warning markers for later-stage, more serious problems? That, of course, was all built upon the belief that each individual patient was different from any other patient and had genetic uniqueness. This was first discussed by Dr. Williams in *The Lancet*, in a classic paper published in 1950 (three years before Watson and Crick talked about the structure of the gene and the double-helix). This was all discussing biochemical individuality. Dr. Williams ultimately derived the term "genetotropic disease." In this landmark paper in *The Lancet*, where Dr. Williams describes and defines genetotropic disease (later reviewed in *Nutrition Reviews* in 1950), he talks about how genetotropic disease describes a condition that a patient has that is of a complex origin and that came about because of long-term nutritional inadequacies (individualized to the patient).⁵ This is seen in such things as increased risk to diabetes, heart disease, arthritis, and a variety of conditions, including complex disorders like alcoholism.

This work was received with great resistance and tremendous push back from the medical and scientific community when it was published. In fact, the genetotropic concept of nutrition (when reviewed in the *Journal of the American Dietetic Association* in 1954) was criticized because it "did not have adequate clinical support."⁶ Over the years, however, we have seen this concept of genetotropic disease become accepted due to a better understanding of the underlying clinical and laboratory science.

In 1953 in *Science* magazine, an article was titled "Genetotropic Disease," authored by Loeffler and Mefferd, in which they said that the concept of genetotropic disease offers a totally new paradigm potential in the origin and explanation for complex chronic disease.⁷ In fact, Dr. Williams, himself, in the classic article, "The Concept of Genetotropic Disease," which was published in 1950 in *The Lancet*, said that individual metabolic patterns could result from genetic uniqueness that is modified by environmental factors, of which the major one is nutrition leading to expressions of different patterns of function.⁸ In a *Proceedings of the National Academy of Sciences* paper in 1949, Dr. Williams and his colleagues wrote about individual metabolic patterns, genetotropic disease, and alcoholism.

Alcoholism: Connections to Genetotropic Disease and the Dopamine Receptor

What we have just heard from Dr. Deth causes me to go back and rethink alcoholism, arousal, attention, and the dopamine receptor. Those of you who are following this in the area of neurology and behavioral psychology are undoubtedly well ahead of me, because you know that there is an ever-increasing body of literature to indicate that dopamine receptor polymorphisms are associated with the risk to various substance abuse problems. People who may have depressive tendencies and/or low arousal tendencies often have dopamine receptor polymorphisms. From what we have learned from Dr. Deth, these individuals may be less able to transfer methyl groups to membrane phospholipids and to respond to

messages of arousal and attention and mood. This is a very interesting concept.

I want you to recall that in 1949 Dr. Williams had the clarity of understanding to propose the concept of genetic uniqueness, although he had no idea of DR4 and he had no idea of molecular genetics post Watson and Crick and he had no idea of the birthing of nutrigenomics that would occur around the turn of the 21st century. He gained this understanding from animal work and the extraordinary studies he had done with nutrition, noting that the modification by environmental factors in the diet were extraordinarily important in determining outcome (phenotype and trajectory of response). Even such things as sugar craving and alcohol craving in animals could be modified by dietary variables that were unique to the individual.

Think about this in the context of what we are seeing in our society today, such as the "*Supersize Me*" fad or the high sugar-high fat diets. Receptors are pleasure centers that can give constant loud messages of joy and of pleasure going to our neurochemical system, and they are varied in they way they are responded to on the basis of the person's own unique biochemistry. But in the end-just like turning up the volume at a rock concert to the point that it causes ear damage-eventually the receptor system goes quiet; it loses its sensitivity. It accommodates for this excessive noise by turning down the sensitivity so you have to go louder and louder with more and more messages of excitement to ultimately get the arousal to be perceived.

What Dr. Williams was talking about with genetotrophic disease is genetic uniqueness not being met by the nutritional intake of the individual and the resulting effect on expression over time-not in a day, maybe not in a week, maybe even not in a month-but over months to years (maybe even over decades). Consumption of a suboptimal diet ultimately leads to suboptimal performance that culminates as a disease. With alcoholism, his principle was that there is something unique in the genes that if not properly modulated in the diet, can express itself as an increasing hunger for alcohol.

Recent Literature about the DR4 Receptor

The literature on the DR4 receptor and alcoholism connection is very fascinating. We know that cortical dopamine receptors genetically seem to be different in alcoholics versus controls, which can be seen in whole-hemisphere autoradiography and cortical function tests in the individual. This was published in *Psychopharmacology* in 2005.¹⁰ We know that if you knockout different types of message signaling systems in the brains of mice it influences dopamine receptor activity. These mice will display reduced ethanol-induced rewarding effects, which alters their hunger for alcohol; this was shown in *Neuropsychopharmacology* in 2005.¹¹ We know there is strong evidence that dopamine receptors are interrelated to the addictive properties of alcohol, and that different polymorphisms appear to be more susceptible to alcohol intoxication and other substance abuse dependencies. These relate, again, to the uniqueness of the individual with regard to their own genes.

The long-term effects of maternal separation on ethanol intake and brain opioid and dopamine receptors was studied in rats. Alteration in dopamine receptor activity and function and alteration in dopamine levels stimulating arousal translated into differing intakes of ethanol (increasing intakes with lower dopamine activation) were associated with time away from mothers.¹² Dopamine receptors and transporters in alcoholics were measured by whole-hemisphere autoradiography in humans, and differences were found between the Cloninger type 1 and type 2 alcoholics.¹³ Some studies have also

suggested that novelty-seeking or extraversion behaviors are associated with alcoholism, but the relationship with Dopamine receptor polymorphisms is not well established.¹⁴

The Relationship between Alcoholism and Nutrition

The concept is that there may be genetotrophic uniquenesses to alcoholism. The question is how does that relate to nutrition? I am, again, going back to Dr. Roger Williams. Remember that his first papers on alcoholism as a genetotrophic disease go back to 1949, well before any of the information in the discussion that we had shared with us by Dr. Deth was even the glimmer of understanding or a hypothesis. Is there any relationship between those nutrients that we have talked about with regard to methylation properties through the DR4 receptor and alcoholism? This would have to do with methionine-homocysteine transference and those nutrients like B12, B6, folic acid, or 5-methyltetrahydrofolate and betaine.

If you do a quick little research review of the literature on homocysteine and alcoholism, you find some very interesting things. Recently in *Epilepsia* in 2006, a paper was published titled "An Assessment of the Potential Value of Elevated Homocysteine in Predicting Alcohol-withdrawal Seizures." This study investigated the observation that alcoholics with elevated homocysteine levels have more difficult withdrawal symptoms.¹⁵

Alcoholism and Plasma Homocysteine Levels

We recognize there are short-term cognitive deficits during early alcohol withdrawal that are associated with elevated plasma homocysteine levels in patients who have alcoholism. This was the *Journal of Neural Transmission* in 2006.¹⁶ We know there is a very high presence of homocysteinemia in chronic alcoholics. One could ask, is that a cause or an effect? Is it an associated variable as a consequence of the alcoholism or does it precede the alcoholism as a co-variable due to alterations in the genetics of methyl transfer reactions?

These are all very interesting questions that I think bear back on what Dr. Roger Williams talked about in the genetotrophic theory of disease and alcoholism being an example of one of those diseases. I have just cited a paper on the prevalence and mechanisms of hyperhomocysteinemia in chronic alcoholics that was reviewed in *Alcohol Clinical and Experimental Research* in 2005.¹⁷

What I would like to leave as a concept for us to consider is that maybe there is more than autism and maybe there is more than ADHD that is associated with what Dr. Deth has been proposing to us: that these are examples of genetotrophic complex situations; that a variety of different chronic conditions that present themselves as not related, one to the other, all may have some connection to this methylation pathway and to unique concepts of genetic transference of methyl groups or methionine synthase and this whole other family of enzymes that are involved in the tetrahydrofolate cycle and methylation as a pathway.

Depression: Connections to Genetotrophic Disease and the Dopamine Receptor

In many individuals, depression is a condition treatable with intramuscular B12 injections and oral, high dose tetrahydrofolate supplementation. A paper in the *Journal of Psychopharmacology* in 2005 talks about the prevalence of low B12 status and low folate status in depressive patients and the use of diets and/or

supplements that are high in folate and B12.¹⁸ This is seen more frequently in individuals having the MTHFR C677T polymorphism that impairs homocysteine metabolism and this is over-represented among depressive patients. The authors talk about oral doses of over the RDA for folic acid (around 800 mcg daily) and B12 (1 mg per day) should be tried to improve clinical outcome in patients with depression. Again, Dr. Williams in his genotrophic concept of disease, talked about this as being an example (depression and alcoholism as genotrophic diseases). Now, some 60 years later, we are revisiting this in light of new mechanisms in the work that Dr. Deth shared with us (methyl transfer reactions and the DR4 receptor and how this relates to possible propensity or sensitivity to melancholia and to lowered arousal and lowered attention).

I think these are quite dramatic examples of where this field of functional medicine is going: to interconnect different pieces of information to try to piece together a detective puzzle that leads to different clinical approaches beyond just treating the individual symptoms, or, what some people call, "chemical incarceration" (giving a drug that will suppress a certain symptom but then makes the individual less than fully functional).

Betaine: An Important Cofactor in the Tetrahydrofolate Cycle

One of the things about this homocysteine connection and methylation that I wanted to add to the story (because we often forget its importance) is the additional methyl donation nutrient called betaine. Betaine is an essential cofactor in a portion of the tetrahydrofolate cycle. As I mentioned, there are a number of different enzymes involved in the tetrahydrofolate cycle (methionine synthase only being one) that all have the potential for polymorphisms. That is one of the reasons that this is a very complex problem to treat because you can have a multiple series of uniquenesses in this pathway that present as the same thing (elevated homocysteine) but have come from differing contributions. Betaine (or so-called trimethylglycine) is a very important cofactor in one of these steps involved with the tetrahydrofolate cycle. It was shown a number of years ago by the Wilkins husband-and-wife-MD team in Australia that people who are non-responsive to B6/B12 and folic acid but have elevated homocysteine may respond to supplemental doses of betaine.

The Effect of Betaine on Plasma Homocysteine

In a paper that was published in the *Journal of Nutrition* in 2006, investigators evaluated orally administered betaine and its effect on both serum betaine and plasma homocysteine concentrations in apparently healthy humans.¹⁹ In this particular study they used graded doses: 1, 3, and 6 grams per day of betaine. The doses were mixed with orange juice and ingested after a 12-hour overnight fast. The volunteers were all randomized into different treatment groups. They found that orally administered betaine had an immediate and dose-dependent effect on serum betaine, so it was well absorbed and could be measured in plasma. Single doses of 3 and 6 grams lowered homocysteine significantly, whereas the 1 gram dose did not. So, it appears as if you need at least 3 grams in order to have a homocysteine-lowering effect if a bolus dose is given. The change in plasma homocysteine was linearly associated with the betaine dose and also serum betaine concentration. Urinary excretion of betaine seemed to increase with an increasing dose. I think we should keep in mind that that is another component in our clinical bag of tools for managing homocysteine and promoting support for methylation reactions. This, again, is trimethylglycine and the single dose threshold in the healthy human population was around 3 grams.

Are Specific Genotypes More Likely to Respond to Vitamin B Supplements?

When we go back to where I started this discussion at the beginning of this month's *Functional Medicine Update*, I talked about the homocysteine-lowering studies with folic acid and B vitamins in vascular disease (the so-called HOPE trials-the Heart Outcomes Prevention Evaluation Trials), published in *The New England Journal of Medicine* and which were negative about the value that B vitamin supplements for cardiovascular benefit. One has to ask the question now, are there specific variant genotypes that are more likely to respond? Are we over generalizing to say that all people with homocysteinemia will have the effects of lowering the homocysteine from B12 and folic acid or B6 alone, or are there variants that are more highly sensitive to this interrelationship and by stratifying the data to those genotypes we might see much more significant interrelationships between vascular disease, homocysteine, and the lowering of homocysteine with folic acid and B vitamins? I don't have the answer to that question, but I am just trying to raise in our minds some observations that are coming from how this literature and these studies are proceeding over time, but always going back, really, to Dr. Kilmer McCully's observations and (preceding that) to Dr. Roger Williams with his genotrophic disease concepts. I think often we tend to want to throw the baby out with the bath water. We find, from one study, that something did not work as we thought and therefore we go back and throw out in our minds the whole conceptual framework without understanding the specificity of that study relative to the framework. The framework of the genotrophic disease concept is as viable and strong today as it ever was and it continues to get stronger with more information that is accumulating related to nutrigenomics and to chronic disease etiology.

I think the homocysteine trials are very important and I think we have a lot to learn about individualization and genotypes that pertain to individual response.

L-Arginine Supplementation: Discussion of a Response to a Study Published in JAMA

I would not have done a complete discussion on this subject if I did not also talk a little bit about the study by Schulman, et al, in the *Journal of the American Medical Association* in 2006, surrounding the role of L-arginine supplementation in vascular disease. You know L-arginine also plays a role (through its influence on endothelial nitric oxide generation) on the homocysteine-mediated effects on the vascular endothelium. You might recall that the Schulmann paper, which describes studies of intervention using oral arginine supplementation failed to show any positive benefit on endothelial function or on cardiovascular risk from arginine supplementation.

This article was titled "L-Arginine Therapy in Acute Myocardial Infarction: The Vascular Interaction with Age in Myocardial Infarction (VINTAGE MI) Randomized Clinical Trial" and it appeared in the January 4, 2006 *JAMA*.²⁰ The clinical study enrolled patients who were at least 30 years of age and within 3 to 21 days of their first ST-segment elevation myocardial infarction (or STEMI). Cardiac catheterization was performed and patients were documented with Q-wave MI, present cardiogenic shock, active acute coronary syndrome, and severe underlying non-cardiac disease limiting predicted life span to less than one year.

If we were took those individuals who were excluded from the study, and also excluded people who had poorly controlled diabetes or renal disease or hepatic disease, and then we looked at the effects that L-arginine had in that inclusion population, it didn't look as if there was any positive influence on vascular function from L-arginine supplementation. However, as you probably recognize, there were a number of questions raised about the study and its conclusions.

Published Comments of Dr. Louis Ignarro

I think the most insightful of these comments were from Dr. Louis Ignarro, distinguished professor of molecular and medical pharmacology and also the Nobel Prize recipient in Medicine and Physiology for the discovery of nitric oxide at the Department of Pharmacology at the University of California, Los Angeles. His response was recently published in the *Journal of the American Nutraceutical Association* in 2006.²¹ In this particular response Dr. Ignarro raised a number of questions about the study and the conclusions derived from it. He says that L-arginine is one of the most abundant amino acids in the diet and adult humans consume 3 to 6 grams of L-arginine daily depending on their dietary protein habits (it is higher in vegetable protein than it is in animal protein). Administering L-arginine is something we do everyday when we eat a normal diet. He goes on to say, however, that therapeutic L-arginine given as a single amino acid can have a very significant positive role in increasing endothelial nitric oxide activity and reducing asymmetrical dimethylarginine levels, all of which have been shown by other studies to have positive benefit (long-term) in the prevention of vascular dysfunction. His question is: Does the absence of an effect with an acute administration of L-arginine post MI negate the potential benefit of L-arginine taken prophylactically to improve endothelial function with individuals who have marginal endothelial dysfunction (as measured by vascular elasticity studies or modest hypertension)? Many studies, as Dr. Ignarro goes on to point out, have demonstrated that L-arginine in humans has a salutary and beneficial effect on those vascular functional parameters, well before acute therapeutic effects as a drug are talked about.

I think this raises some interesting questions about the role these nutrients play in medicine. We are really talking more about functional interventions when we are talking about things like ADHD, or alcoholism, or vascular/arterial stiffness; these are functional parameters (well before acute pathology) that relate to fundamental underlying translations of genetic uniqueness into phenotypic outcome (into the function of the individual). I believe this model is perfectly compatible with a pathology-based model because there will always be need for managing a patient who has a demonstrable pathology using acute intervention strategies. These may be new-to-nature molecules that are derived from the wonders of pharmaceutical science or surgery or many other new technologies that are being developed, including biotechnology. But in the same breath, there is also the need for ways of intervening early through functional improvement using those indicators of suboptimal translation of genes into phenotype. That is where the story that Dr. Deth has been sharing with us on this issue may play such an important role in the framing of this new medicine.

Thanks for being with us. We are going to have some questions and answers to talk about in this particular issue.

Questions and Answers

I would like to finish up this month's *Functional Medicine Update* with a few questions that have been raised by our listeners that I think are clinically pertinent and probably of general interest.

The first relates to magnesium status and how we evaluate it and what some clinical signs and symptoms are. Dr. Sidney Baker talks about "zips" and "zaps" that are associated with magnesium deficiency. These are funny little pains-like short circuits in your neurological system-and you get spontaneous muscle cramping. These things are not as obvious as pathophysiologicals, but they are early-warning clinical markers of magnesium insufficiency.

A more clinically relevant issue, mitral valve prolapse, has a very interesting (and not obvious) connection to magnesium insufficiency. Idiopathic mitral valve prolapse refers to the systolic displacement of one or both mitral leaflets into the left atrium, with or without mitral regurgitation. It is not uncommon, it is seen more frequently in women than in men, and it usually appears to be a benign condition and even capable of recovery.

In a minority of cases, mitral valve prolapse may predispose to complications. Some evidence has accumulated suggesting genetic heterogeneity, and possibly some SNPs (Single Nucleotide Polymorphisms), that are related to susceptibility to this condition. It appears as if there is a magnesium connection to this condition and it may be connected through specific SNPs that make this condition more prevalent in certain genotypes. This has to do with what might sometimes be called "latent tetany" (tetany being an example of an acute magnesium deficiency).

There was a review paper on this subject published in *Magnesium Research* in 2005 that titled "The Importance of Magnesium Status in the Pathophysiology of Mitral Valve Prolapse."²² In this article, the authors write about the fact that there are now a variety of both epidemiological and intervention trials that seem to indicate that hypomagnesaemia is associated with increasing incidence of mitral valve prolapse. This question of hypomagnesaemia is an interesting question in its own right. Are we talking about low serum levels of magnesium (the most standard clinical evaluation that is used for detecting magnesium insufficiency), or are we talking about intracellular magnesium insufficiency (which deals with something like red cell magnesium, if the test was done right and the red cells didn't leak and lead to partial hemolysis that causes alteration in the test values), or are we talking about a functional test for magnesium (which is to do a magnesium loading test where you administer oral or intravenous magnesium and then you look at the level spilled in the urine in 24 hours? Generally if a person is saturated with magnesium, the loading test will show significant excretion in the urine of that magnesium load (over 80{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} will be excreted in 24 hours). If a person is magnesium depleted, then obviously their cells will pick up and incorporate that magnesium and so their urinary spill may be very nominal over the next 24 hours (10-15{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} spill). The loading test has been considered by many to be a better functional test for magnesium status as it pertains to cellular levels. The intracellular erythrocyte magnesium is kind of considered the second-level test for detecting magnesium status. Lastly, is the serum magnesium test (which has limited value because you are not sure exactly what you are measuring in that magnesium is historically an intracellular element as contrasted to, say, sodium and calcium, which are extracellular). The assessment tool that best clinically correlates with mitral valve prolapse is to use either red cell magnesium or the magnesium loading test; it doesn't seem to correlate too closely with the serum magnesium level (that may be more related to frank magnesium deficiency, when you are getting into tetany and more severe magnesium deficiency symptoms).

If you look at this magnesium connection, we are really talking about lymphocytic magnesium being very important. Lymphocytic magnesium is indirectly related to erythrocyte magnesium, which is then correlated with the relative function of cardiac activity and the mitral valve function. There is a published paper that discusses magnesium deficit in lymphocytes as part of the mitral valve prolapse syndrome. This paper appeared in *Magnesium Research* in 2004.²³ There is ever-increasing evidence to suggest that hypomagnesaemia, as measured by these cellular functional tests (either erythrocyte magnesium or the magnesium challenge excretion test) are very good correlates with the relative risk to mitral valve

prolapse. If you are examining a patient and, when doing your cardiac evaluation, it turns out you have a sticky valve or something that is going on that suggests mitral valve prolapse, you might consider doing a magnesium status evaluation either with the red cell magnesium or the magnesium loading test.

The other question that has been raised, and I think for very good reason, is the question concerning what appears to be the complex (and maybe counterintuitive) results of the studies that have been recently published on homocysteine and the role that vitamin supplementation with folic acid and vitamin B12 have in altering cardiac risk in patients with hyperhomocysteinemia. As we will discuss in more detail in this issue of *Functional Medicine Update*, the HOPE 2 Trial results were recently published in *The New England Journal of Medicine*. This trial focused on homocysteine as a biomarker for cardiac risk and the role that vitamin B12 and folic acid supplementation has in modifying that relative risk through the lowering of that biomarker.

The conclusions of the study were very disappointing for those of us who felt that folic acid and B12 do play salutary and beneficial effects in hyperhomocysteinemia and lowering cardiac risk because the data did not demonstrate that; it did not say that there was any real advantage. So, what about these studies? I have been in conversation with my colleague, Dr. Dan Lukaczer, about this and I want to share our joint view on this topic because we have had a chance to exchange some thinking. If you look at the HOPE 2 Trial results published in *The New England Journal of Medicine*, there are a number of things about these studies that raise some questions. I want to be cautious about not always being critical of a study that is negative to our belief because sometimes a negative means a belief was wrong, it does not mean the study is wrong. There are, however, some questions about the design of this study.

The patients who were recruited for the study had initial homocysteine levels that averaged around 12 ng per milliliter, which many investigators might think is not high enough to really demonstrate the pathophysiology of homocysteine (more around 15 or higher). Maybe the average levels were lower than desirable. Second, the reduction in homocysteine after intervention was not really down to the level that most of us would like to see homocysteine reduced to (Dr. Kilmer McCully talked about this and it was somewhere around 7 ng per milliliter). The average lowering in these subjects in the HOPE 2 Trial was between 9.6 and 9.7, which we might consider not low enough. Maybe they did not start high enough, and maybe at the end of the study they did not end up low enough on the intervention trial. This may have been because they didn't use high enough doses or the prolongation of the therapy was not extensive enough, or maybe they had a collection of different genotypes, some of which were more responsive and some less and so they actually averaged out (all questions yet to be answered). Another (and probably more interesting) part of this story is that this study used people who had already-existing cardiac disease. These certainly were not healthy people and this was not a prevention trial (not to the extent of primary prevention, this was secondary prevention).

The study was 2 to 3 years in duration. If a person has already had an MI and they have a lot of pathophysiology going on, is it realistic to expect that in 2 to 3 years of intervention (when they only lower their homocysteine down to a modest level) that they are going to get marked improvement when they have already had injury to their arterial endothelium and their vascular function? That is a question for which I don't have an answer. I think raises some questions about the extrapolation (or conclusions) of this data to apparently healthy people in an earlier stage of cardiac dysfunction or endothelial dysfunction, and what role intervention with folic acid and B12 might have in them. I think we still have not really answered the question about prevention versus treatment. I would consider secondary prevention to really

be treatment because we have ongoing pathophysiology. If we are really dealing with an intervention based upon a sick population, the question is what level of intervention is required for promotion of function (in that their dysfunction may be multi-phasic in origin because one thing begets two begets three begets more than that when systems start to fall apart to pathology)? Maybe you cannot just put one block in the dam and have it all focus on health because you've still got other things going on in the pathophysiology of the arterial wall.

I think we need to keep in mind the HOPE 2 Trial as we start looking at how therapy designed to lower homocysteine is applied. In the course of this month's FMU we are going to be talking about some other additional elements (beyond folic acid and B12) that you, as a clinician, should be aware of. These elements include trimethylglycine or betaine intervention, because we recognize that this is another important nutrient for the lowering of homocysteine and the improvement of vascular function (this is work that we cite). We will also address pyridoxine (vitamin B6).

We are starting to look at variables that relate to this complex array of polymorphisms associated with the folate cycle, to modify homocysteine metabolism and to produce improved arterial function. I think you will find that there is an "aha" beyond that which the HOPE 2 Trial talked about, and that is what role do these methylation functions have (in which the surrogate marker for their evaluation is homocysteine elevation) on cellular function across many diseases, independent of ICD-9s? It may be that homocysteine is a phantom surrogate marker. It may be that it is only part of the story or the tip of the iceberg for broader implications of the methylation story.

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