Health Condition: Pediatric Concerns & Autistic Spectrum Disorders

“I was at a meeting at the Institutes for the Achievement of Human Potential, where I have been a member of the scientific advisory board for many years. It is an institute in Philadelphia founded by Glenn Doman and his colleagues 50 years ago to work with brain-injured children and their families. I met a very amazing woman, Laurette, who was the mother of a daughter who had leukemia. This was probably not your average mother in terms of her advocacy—or the lengths that she would take her advocacy—for her daughter. Her daughter, who was receiving repetitive treatments with anti-folate chemotherapeutic drugs (I think methotrexate, and other medications like that), ended up having seizure disorders, which is not an uncommon secondary side effect from these treatments. Laurette asked the attending oncologist and physicians if this side effect could be reduced or removed. They didn't have a lot of clues about this, so she went on her own into the literature world (being a sleuth for information), and eventually hit on Dr. Jill James' work. This work has to do with folate, the methylation pathways, homocysteine, and all these variables. And so Laurette came back to the people at the hospital with reams of information. They said it was very interesting intellectual stuff, but it didn't really relate to the practice of clinical medicine and her daughter. Laurette made a big case of this and her advocacy was undaunted. She said that her daughter should be treated with folic acid and other methylating nutrients and that this would reduce the seizure disorders. So the debate went on and on. The products Laurette was requesting were not available in formulary, so the hospital staff couldn't really prescribe them, and so Laurette fought through that to get them on formulary. The long and short of it was that through her advocacy and through mobilizing the work of Dr. James and others, Laurette was able to better manage these problems with her daughter in terms of seizure disorders. Needless to say, Laurette is quite an advocate.”

—Jeffrey Bland, PhD
January 2007

Big Medicine, Small Children

Children are the most vulnerable members of our society, and when they need medical attention, either for acute illness or for chronic developmental issues, who but their parents or guardians can better advocate for their health and wellness? Chronic childhood health issues are on the rise. This includes allergy, atopy, and asthma, as well as learning and developmental issues, such as Attention Deficit Hyperactivity Disorder (ADHD) and Autistic Spectrum Disorder (ASD). Are the numbers really rising, or has diagnosis just improved? This issue is one of the most significant debates in the field medicine, one that is at the forefront of medical controversy, medical interest, media attention, and parent activism. It has all the trappings of one of the great social issues and historical medical movements of our time.

Allergy, Atopy, & Asthma

Many theories based on epidemiologic or experimental data have been advanced to explain the increased incidence of asthma and other atopic conditions in our society. Those factors include improved hygiene, changes in diet, changes in intestinal microflora due to increased use of antibiotics, altered patterns of infant feeding, greater exposure to allergens, obesity, reduced physical activity, and

changes in the prenatal environment. This may be an example of a high genetic susceptibility, coupled with a disruption through environmental triggers.

Atopic disorders have to do with the balance between the T helper cells, the Th-1 and Th-2 expression of these intercellular mediators—the cytokines, the interleukins, the chemokines, which then alter function at a distance in the body and are associated with allergen-specific IgE and eosinophilic inflammation. According to the hygiene hypothesis, infection with virus and perhaps other intercellular organisms at an early age influences the developing immune system and changes the way these Th-1 and Th-2 mediators are produced throughout the rest of one's life.

Approximately 4 to 8% of children and 1 to 2% of adults have a true food allergy. These are individuals in which there is a definite demonstrable presence of IgE antibody. But a much higher percentage of people report sensitivity to certain foods resulting in physical symptoms. Vincent Marinkovich, MD, now deceased, maintained a private practice specializing in allergy and immunology for many years while also serving as an associate professor of medicine at Stanford University Medical School. In a 1999 interview with Dr. Jeffrey Bland, Dr. Marinkovich explained the difference between food allergy and food hypersensitivity this way:

"From my own clinical experience I feel the immune system plays a far greater role in producing symptoms in patients from the foods they eat. But these patients are not identified by skin testing, which is the procedure traditional allergists use in making their diagnoses. By far the great majority of food reactions are mediated by other-than-IgE mechanisms, and many of them are mediated by other immunological mechanisms, which could fall under the category of allergy, except that by definition allergists want to exclude those. I call them hypersensitivity reactions… The immune system is far more active in the body than simply being involved in IgE reactivity. A number of other immunoglobulin antibodies can play a role, such as IgG, IgM, or even IgA. They can all play a role in the kind of disease one might have or the symptoms one might have from eating a specific food.

To determine whether this kind of a process is active, one needs only to measure the one immunoglobulin that is most likely to be a marker of a generalized immune response to a food. For example, if a patient is showing immunological reactivity to milk, it may be an IgE reaction, in which one has to do a blood test that measures IgE, or one can do a skin test. If a clinician has a non-IgE-reacting patient, he or she has to measure the marker IgG. And measuring IgG simply tells you there's a generalized immune response to that food. A number of players may be part of the immune armamentarium in causing the symptoms. But the single best marker of that is a blood test that measures specific IgG antibody."

Dr. Marinkovich further explained the physiological reaction to food hypersensitivity:

"When you eat a food to which you have significant antibody levels, and if that food can go across the mucosa, the lining of the intestine, without being completely digested, then it is able as a large protein or polypeptide to combine with your IgG or other antibody. In doing so, it forms an immune complex. That's a normal way the body deals with foreign materials. It forms immune complexes. But then these immune complexes float around in the body just for very brief periods of time, because they are very inflammatory.

The body knows it must get rid of them by extracting them from the bloodstream. It has a large number of cells that are key to removing immune complexes from the circulation. This process happens extremely rapidly if the complexes are large enough. In fact, one pass through the liver or through the spleen and the immune complexes are largely removed in a normal individual. The problem becomes a more disease-producing one when these scavenger cells, or macrophages, become filled, when they are no longer able to attract the immune complexes out of the circulation, and the complexes continue to circulate. Then they begin to form a pattern of their own based on their own inherent chemical properties. They tend to deposit in certain tissues, such as the joints, the walls of the blood vessels, or the kidneys. Wherever they deposit, they create inflammatory conditions that lead to illness focused upon that particular organ."

He also explained the difference in clinical manifestations between children and adults:

"In the very young infant you will often get projectile vomiting because of the swelling of the gastric mucosa blocking the outflow track to the stomach. Therefore, when the stomach contracts the infant tends to vomit upward with some force, based on that gastric contraction. Nausea symptoms and colic in young infants are all part of the spasm that is induced by the inflammation in the gut wall when antibody and antigen come together. That is especially true in infants in whom there is a lot of leakiness of protein materials across the gut wall, which doesn't occur later in life. As children get older you see more nasal congestion and eustachian tube dysfunction. The middle ear no long can clear fluids, and patients develop recurrent ear infections or can't hear as well. As individuals become adults, more sophisticated and cognitive differences become more important. There are also other symptoms of system failure. The joints become more swollen, for example, and you see more problems with joints as you get into older age groups. You see chronic diarrhea and headaches, such as migraine headaches, almost all of which are caused by immune complexes from immune reactions to various foods. You do see these changing patterns, depending on age."

In 2004, an article was published in the *Journal of the Royal Society of Medicine* indicating that in the past three decades, we have witnessed a spectacular increase in the prevalence of asthma and allergic disease worldwide, especially in countries with a western lifestyle.[1] In the International Study of Asthma and Allergy in Children, the highest prevalence of asthma was found in Australia, New Zealand, and the United Kingdom, where, in 2003, more than 20 percent of children aged 13-14 were reported to have symptoms of asthma. That is one in every five children. By contrast, in central Africa, and central and eastern Europe and China, the prevalence of childhood asthma was less than 5 percent. This increase in prevalence in the UK, New Zealand, and Australia has occurred in the last 20 years and is even greater than it was during periods of great pollution and environmental soot in London that led to all sorts of respiratory problems.

Clues to environmental factors that drive the rising trends may be revealed from a closer look at disease mechanisms. With the recognition that gene-environmental interactions are critical to the understanding of the pathogenesis of allergic disorders such as asthma, there has been a major focus on the immunological and inflammatory mechanisms that underlie the origins of allergy and its progression to allergic inflammation. People are starting to look at what environmental factors cause this dysregulation of the immune system.

Some things have emerged from this research. First of all, there is a strong, socioeconomic gradient. Many higher socioeconomic categories of individuals seem to have increasing risk to these atopic disorders. There are fewer cases of allergy in large families, less in last-born siblings, less in rural than

urban environments, less in developing countries, and less related to gastrointestinal infections. There appears to be some kind of immune resistance that results from GI infection, e.g., hepatitis, toxoplasmosis, or *H. pylori* infection. There is less allergy in children who attend day care centers, meaning they are getting cross exposure and developing almost an immunity. That has led to what has been called "The Hygiene Hypothesis." If we are overly conscious of hygiene and do not allow children's immune systems to be properly stimulated, they will not develop antibodies and resistance against allergenic agents. That makes them more susceptible to asthma and allergy in their adolescence. We need to modulate the balance between the Th-1 and Th-2 sides of the immunological system.

Attention Deficit Hyperactivity Disorder (ADHD)

In 1973, Benjamin Feingold, MD, a pediatric allergist practicing in California, proposed that diets high in food coloring, food additives, and salycilates were the source of hyperactivity disorders in certain children. During the 1950s, supermarkets began selling shelf-stable foods developed by the food industry that were sweet, colorful, and bursting with synthetic flavors. By the 1970s, a generation of kids had been raised on these artificial foods. Dr. Feingold had the audacity to say that some of these food materials and ingredients might be producing neurochemical changes in children with genetic susceptibilities and causing behavior disorders. His views and his advocacy for a diet free of salicylates, artificial colors, and artificial flavors created controversy and a groundswell of antagonistic response from individuals with vested interest in the status quo. In the decades since Dr. Feingold's hypothesis, we have learned more about brain biochemistry, NMDA receptor sites, glutamate sensitivity and neuron cytotoxicity. We are starting to see genetic variants that can respond to some of these substances in our diets. These chemical agents can induce functional changes.

In more recent years, a hypothesis has emerged that long-chain polyunsaturated fatty acids play a role in modulating function in children with ADHD. This hypothesis has not been confirmed, but evidence from some double-blind, placebo-controlled trials points to a benefit in supplementing these children.[2,3] Gamma-linolenic acid (GLA), an omega-6 fatty acid, is a precursor to the 1-series prostanoids, antiinflammatory, anti-self-proliferative, anti-platelet adhesive. GLA has been shown to be a useful therapeutic tool for modulating atopic eczema. The use of polyunsaturated fatty acids to promote skin integrity, immunochemical defense, and lowered inflammatory potential seems to be gaining credibility.

Some of the most recent research into molecular pathways that may underlie cognition and behavioral changes in the case of conditions such as ADHD focus on the neurotransmitter dopamine, and most specifically on the D4 dopamine receptor. The D4 dopamine receptor is the only member of the dopamine family that is able to transfer methyl groups (that it receives from the folate pathway). This is a process that was discovered by Richard Deth, PhD, a professor of pharmacology at Northeastern University, and his colleagues. The D4 receptor is the only one of the G protein-coupled receptors that has methionine residue, and that is the critical chemical location necessary in order to be able to carry out this process.

Dr. Deth describes the ramifications of this discovery in an interview with Dr. Jeffrey Bland in 2006:

“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.”

He continued to explain the connection of the D4 dopamine receptor to attention:

“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 conformation 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 Neubrander. 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.”

The work of Dr. Deth and his colleagues can be summarized this way: These are 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 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 is a very dynamic dance that has a lot of genetic underpinning, and some environmental factors can influence this.

While research continues, children are diagnosed with ADHD daily, and many parents and healthcare providers turn to pharmaceutical interventions to manage symptoms. If we look at the drugs used to treat ADHD, it is a very interesting constellation. On February 9, 2006, the Drug Safety and Risk Management Advisory Committee of the FDA voted, by a narrow margin (8 votes to 7), to recommend a black-box warning description (for cardiovascular risk) be placed on stimulant drugs used to treat ADHD.[4] This decision came more than a decade and a half after these drugs were first prescribed for our youth. The drugs under review were primarily amphetamines (Adderall and other brands) and methylphenidate (Ritalin, Concerta, and other brands). These drugs are broadly classified as sympathomimetic amines and have very active effects, not only on the central nervous system, but also the cardiovascular system. They substantially increase heart rate and blood pressure, and in placebo-controlled trials (when administered to adults) they increase systolic blood pressure by 5 mmHg. Similar effects were found across all the drugs in these families. The FDA advisory committee heard testimony indicating that 2.5 million children now take these stimulant drugs for ADHD, including nearly 10 percent (or 1 out of every 10) of ten-year-old boys in the United States.

Autistic Spectrum Disorder (ASD)

The autistic spectrum consists of a group of developmental disorders with lifelong effects. They have in common a triad of impairments in social interaction, communication, and behavior, often with narrow and repetitive patterns of behavior. The triad can occur on its own, but it is often accompanied by other features. It can be found together with any level of ability, from profound general learning disability to average or even superior cognitive skill in areas not directly affected by the basic impairments. It can occur with any other physical, psychological, or psychiatric condition. It is, therefore, sometimes a wastepaper basket diagnosis for certain types of neurological, physiological, or whole organism performance patterns. A number of interrelated conditions sound like autism. ICD10 subgroup classifications include atypical autism and Rett's syndrome. The F84.3 is another childhood disintegrative disorder. F84.4 is an overactive disorder associated with mental retardation and stereotyped movements. F84.5 is Asperger's syndrome. F84.8 includes other pervasive development disorders. F84.9 is an unspecified pervasive developmental disorder. This is an area of nonspecific definition. It is a series of syndromes that interrelate with one another, with altered communication, altered imagination, poor social interaction, and altered behavior with narrow repetitive patterns.

The etiology of the autistic spectrum is a big topic, and it is surrounded by controversy. A review article on the autistic spectrum in *The Lancet* describes the suggested known etiology, or the etiology that is

respected by traditional neurology.[5] When we look at the way the brain can be altered in its function, however, we come back to the concept of genetics and environment.

Martha Herbert, MD, PhD, is an Assistant Professor of Neurology at Harvard Medical School, a Pediatric Neurologist at the Massachusetts General Hospital in Boston, and an affiliate of the Harvard-MIT-MGH Martinos Center for Biomedical Imaging, where she is director of the TRANSCEND Research Program (Treatment Research and Neuroscience Evaluation of Neurodevelopmental Disorders). In 2005, Dr. Herbert authored an article titled "Autism: A Brain Disorder, or a Disorder that Affects the Brain?"[6] She began this article with an expanded explanation of autism based on her own research and clinical experience:

"Autism is defined behaviorally, as a syndrome of abnormalities involving language, social reciprocity and hyperfocus or reduced behavioral flexibility. It is clearly heterogeneous and it can be accompanied by unusual talents, as well as by impairments, but its underlying biologic and genetic basis is unknown. Autism has been modeled as a brain-based, strongly genetic disorder, but a series of emerging findings and hypotheses support a broader model of the condition as genetically influenced and systemic. These include imaging, neuropathology, and psychological evidence of pervasive (and not just specific) brain and phenotypic features; postnatal evolution and chronic persistence of brain, behavior, and tissue changes (e.g., inflammation) and physical illness symptomatology (e.g., gastrointestinal, immune, and recurring infection); overlap with other disorders; and reports of rate increases in improvement or recovery that support a role for modulation of the condition by environmental factors (e.g., exacerbation or triggering by toxins, infectious agents, or other stressors, or improvement by treatment). Modeling autism more broadly encompasses previous work, but also encourages the expansion of research and treatment to include intermediary domains of molecular and cellular mechanisms, as well as chronic tissue, metabolic and somatic changes previously addressed only to a limited degree."

In 2006, Dr. Jeffrey Bland had the opportunity to interview Dr. Herbert about her research, part of which involved close analysis of the actual brain structure of individuals diagnosed with autism:

"The first thing we saw was that these brains are bigger. We saw an overall volume increase, and this is the most replicated finding in autism research. I have a review of this in the October 2005 issue of *Neuroscientist* titled 'Large Brain in Autism: The Challenge of Pervasive Abnormalities.'[7] We have been oriented toward looking for modular changes to explain modular behaviors, but here, we have a widespread change. We found that the largest brains existed in high-functioning autism (first identified by Pauline Filipek, who is now at the University of California Irvine), compared to controls. Low-functioning autistic brains in our sample weren't as big as the high-functioning brains were, but they were still bigger than the brains of children with comparable mental retardation but without autism. We also found that developmental language disorder (DLD) brains were larger than normal. Nobody had ever measured the whole brain in DLD; everybody had been looking at the language areas, presuming that a language disorder is specific to language parts of the brain. We're now finding out that even developmental language disorder is systemic. These children have a lot of non-language-related neurological problems--clumsiness, and EEG/ERP abnormalities in a variety of things, as well as a possible association with autoimmune abnormalities, in the children or their close relatives in some cases. We found that there was a tendency for the brains to be bigger, and a seemingly countervailing tendency for mental retardation to bring them down a little bit.

Significance of Brain White Matter

In trying to find out what was making the brain bigger, we found that the predominant thing was the white matter. White matter makes up 30 percent of the total brain volume (cerebral white matter), but it makes up 65 percent of the volume increase. That's interesting, because most people don't think of white matter; they're into neurons, and white matter is just supposed to be there to get neuron signals from one neuron to the next one. Actually, it's not looking that way anymore. White matter has become interesting in a variety of neurodegenerative disorders, as well as in children's disorders. The thing about the white matter is, is it all of the white matter, or is it just some of the white matter?

White Matter Parcellation

We have a technique in our laboratory called white matter parcellation. We divide the white matter into zones related to the tract architecture. This was developed for scans where you can't see the fiber tracts, as new diffusion imaging allows you to do. We found that the white matter that was contributing to the enlargement was what we call the radiate--the outer white matter (including the 'corona radiata') tucked under the gyral folds. This predominantly includes short connections between parts of the cortex that are both close to each other and within the same hemisphere--not the longer tracts, not the corpus collosum that connects the two hemispheres, not the long tracts that go down to the body, but predominantly, the shorter corticocortical tracts. This is also the area that myelinates latest--myelination starts deeper in the brain.

We also found that the later an area myelinated, or the longer it took to myelinate, the more it tended to be larger than in the brains of the controls. We felt that we had found some sort of 'archaeological footprint' of something that happens in time, which changes and becomes more intense over time.

The last thing we found was a rather large-scale shift, not away from leftward asymmetry, which was the same in comparing autism, developmental language disorder and controls; but instead an addition of a lot more areas that were rightwardly asymmetric, particularly in the higher order association areas--that is, the areas that are most highly connected up with other multiple parts of the brain. That is interesting, because other people are finding a lot of right hemisphere problems in autism."

The white matter of the brain has the glial cells, which is like the brain's immune system. A hypothesis has been made that autism may be associated with a neuroinflammatory condition. One of the many articles on this subject appeared in the *Annals of Neurology* in 2005 and was titled "Neuroglial activation and neuroinflammation in the brain of patients with autism."[8] In their 2006 interview, Dr. Bland asked Dr. Herbert about the connection of neuroinflammation with autism and she said this:

"For those of us who were already aware of the recurrent infections in these children--the eczema, IBD, and the multiple (not always consistent, but definitely multiple) measures of inflammatory dysfunction--this paper was pivotal, because to see microglial and astroglial activation in the brains of those from five years to 44 years old, and inflammatory cytokine and chemokine profiles in both brain and CSF, says that we're dealing with a chronic disease. The Vargas-Zimmerman-Pardo paper was a breakthrough in identifying these things in the brain and not just 'peripherally.' There's another abstract by Perry and Salomon that reports finding carboxyethylpyrrole, which is a lipid peroxidation marker, in every autistic brain they've cut.[9] This means that what we're looking at in autism is something different than what had been thought before, and it's consistent with what functional medicine is about. Pardo and Vargus say that this is an innate immune reaction in the brain, whereas it's been pointed out that there's both innate and adaptive immune reactivity in the gut. It raises the question of interaction among multiple systems of the body. First of all, it's a chronic disease. Second, it could be environmentally mediated. Third, it's multi-system."

Could autism result from toxic exposures? This is another hypothesis that has been widely reported. Dr. Herbert on this subject:

“Most toxicological studies have been done with high-dose exposures. When you have a high-dose cytotoxic exposure, enough to directly knock off a cell, you're going to get a hole in the brain. A few years ago, when I would discuss these things with my colleagues, they would say that autism couldn't have anything to do with toxins, because if it did, the brains would be smaller. The brains in autism are bigger, so it's got to be something else. But there is so much research going on now having to do with low-dose and multiple toxic exposures. Let's talk about low-dose exposures.

At low doses, chemicals can have biomimetic effects. They can act like other signaling molecules and confuse things. If that happens during development, one could experience changes, not like a hole in the brain, but rather widely distributed, involving changes in scale and proportion. That's the kind of thing I've been measuring. These changes are not hugely dramatic because, first of all, there's not a whole lot of tolerance in the brain. You can't wildly change the size of something in the brain without causing an enormous problem. We need to understand that when we measure volume changes in the brain, they can be subtle. I think the issue is that people have been very much involved in looking at brain behavior relationships, and they've assumed there's some kind of different genetically determined developmental trajectory and that the brain tissue is basically healthy--they've assumed that the brain is just wired differently, or it has different neurotransmitters. If we're now looking at chronic tissue changes, we could have altered developmental trajectories or subtle tissue changes, such as chronic mild inflammation, that could also change things at a subtle level, but wouldn't necessarily be confined to the neural systems that govern any particular behavior. They would just be an overlap between the tissue-based pathology of inflammation or oxidative stress and the behaviorally related neural systems that happen to be in the same territory.”

How should clinicians best approach the treatment of children with autism? Dr. Herbert—a practicing clinician as well as a researcher—also provides valuable insight on this subject:

“To zoom in from 50,000 feet, you'd want to take care of predominant biomedical imbalances in inflammatory oxidative stress and other pathways, and you need to do behavioral intervention with these children, because they need a lot of reinforcement and systems remapping to function. That's the outermost level. In order to figure out which kind of problem is predominant, you need to do further studies and history-taking.

On any clinic day, I'll have some children with diarrhea and other children with no diarrhea; some with regression, some with no regression; some who had 30 ear infections, and others who had none. You need to go through that with some kind of cultivation of biochemistry and immunology, and sort out what the most likely initial target would be that would give you leverage. Also, you can't just do one thing at a time. There are a lot of things going on that are mutually co-modulating and mutually reinforcing. If they were mutually reinforcing in a bad way, you'd want your treatments to be mutually reinforcing in a good way.

Beyond that, there's a lot of material online for the functional medicine approach at the Autism Research Institute. Hopefully, there will be more and more material coming along that will reflect some kind of integration of behavioral and biomedical approaches. There's a textbook, published by the Organization for Autism Research (OAR) on evolutionary approaches to neurodevelopmental disorders, which does some integration of behavioral and biomedical approaches (*Neurobehavioral Disorders of Childhood: An Evolutionary Perspective.*[10]) Hopefully,

we'll see more of that."

The Transsulfuration Pathway: Could it be the next step forward in brain chemistry research?

The importance of methylation in the human body has already been mentioned in connection with Dr. Richard Deth's research on ADHD. Methylation, the addition of a methyl group to a substrate or the substitution of an atom or group by a methyl group, is involved in modification of heavy metals, regulation of gene expression, regulation of protein function, and RNA metabolism.

Sandra Jill James, PhD, a researcher at the Arkansas Children's Hospital Research Institute in Little Rock, Arkansas, has been studying the transsulfuration metabolic pathway for her entire career. Her research initially began with cancer, but now spans many areas related to this pathway, including the central role it may play in the etiology of autism. Dr. James was interviewed by Dr. Jeffrey Bland about her research in 2007:

"This pathway has been my life. It has taken me on a wonderful and fascinating journey. The transmethylation pathway is basically the methionine cycle. What we did is take the methionine cycle through S-adenosylmethionine to S-adenosylhomocysteine to homocysteine. Then we took it down further.

If you take from homocysteine, now you enter the transsulfuration pathway. Most graphs (if you look at the diagrams and papers) will end at cysteine. What we did is say, 'Wait a minute. We need to go all the way down to glutathione.' That was really illuminating for us. What we have looked at in Down syndrome and cystic fibrosis and now, most recently, in autism is how the transmethylation pathway interfaces with the transsulfuration, taking it all the way down to glutathione.

This has been really fascinating to us because there is a lot of regulation that intersects those two pathways. For instance, methionine, which is at the top (it is the product of methionine synthase), is the essential amino acid. What the methionine cycle is basically doing is it is a clever way the cell has to recycle or conserve this essential amino acid. It's basically through methionine synthase and the methyl group from 5-methylfolate. It's a way to keep that methionine level high and that's critically important for the viability of the cell, for not only protein synthesis, but these essential methylation reactions because methionine then leads to S-adenosylmethionine (SAM), the major methyl donor for a multitude of methyl transferase reactions-essential DNA methylation, RNA methylation, protein methylation, phospholipid methylation, creatine, and neurotransmitters. S-adenosylmethionine is absolutely essential to keep that up, which requires the methionine.

The methionine cycle is keeping methionine up, which then feeds to this essential methyl donor. Once it gives up its methyl group, it then becomes S-adenosylhomocysteine (SAH), which is then rapidly hydrolyzed to homocysteine. The next step is from homocysteine down through the transsulfuration to cysteine. Cysteine, recall, is the rate-limiting amino acid for glutathione synthesis.

Now if we go back up to the methionine cycle, the S-adenosylmethionine is an important regulator of CBS (cystathionine beta-synthase), which is the enzyme that pulls homocysteine down transsulfuration. When it is high, it upregulates CBS (when SAM is high you get an upregulation, which pulls homocysteine down to glutathione, which is good). There is that interaction, then, through SAM levels, of regulating the transsulfuration pathway. The other important point here is that methionine, through this pathway, down through CBS and transsulfuration, provides 50% of the cysteine for glutathione synthesis. That transmethylation-transsulfuration pathway is not only

important for methylation reactions, but also as a precursor to get methionine all the way down to cysteine, and that is what leads us into glutathione. We go from methionine being high, and it is going to keep cysteine levels up. Cysteine is the rate-limiting amino acid for glutathione synthesis, and so now the whole transmethylation-transsulfuration works together, and that is actually reciprocal. When glutathione needs are high, transsulfuration is upregulated so that the SAM and homocysteine and the cysteine levels are pushed down that pathway so we can fortify the glutathione levels, which are so important for a multitude of viable options for the cell, including what we call the reducing environment inside the cell (that is what allows multiple redox-sensitive enzymes to work well). You have got to have adequate high levels of glutathione in the cell so that those enzymes are redox functional and active. It is important for the integrity of the cell membrane, for membrane signal transduction, and for gene expression.

Glutathione is an absolutely fascinating molecule. It is important, as you know, as a free radical scavenger. It is well known to be the major antioxidant, or free radical scavenger, inside the cell. It is also important for detoxification; this is less known but equally important, and brings in an environmental aspect. Glutathione is actually a tripeptide: glycine, cysteine, and glutamate. It is the cysteine (and remember, that's the rate-limiting amino acid for glutathione synthesis) component in the glutathione and, specifically, the SH, or thiol group, that's the active part of glutathione; it donates that hydrogen from the SH, or sulfhydryl group. Well, that sulfhydryl group, on glutathione, is a magnet for heavy metals. And, again, we are bringing the pathway now to an environmentally important pathway. That SH group will bind mercury, lead, arsenic, cadmium; it is a magnet for heavy metals. Once bound by glutathione, that heavy metal now is inactive (it can't damage the cell). So glutathione is an important detoxification mechanism for the cell, and that conjugant of glutathione, then, is further metabolized and is excreted from the body in the bile and also in the urine. It is not only the major detox mechanism, it is the way that the metals are excreted, and it is actually the natural chelator of the body, if you want to think about it that way. We have sort of taken our transsulfuration down to being very interested in the glutathione and what we call redox ratio."

How does this very complicated science involve studying children with brain disorders? Dr. James explains, and again we'll hear about Laurette, a mother who would go to essentially any lengths to be a health advocate for her ailing daughter:

"A colleague called who was involved in Down syndrome research, and pointed out that the cystathionine beta-synthase gene is on chromosome 21, and it overexpressed, obviously, because there are 3 copies of CBS in children with Down syndrome. This perked my interest because, of course, that's part of our pathway (and, again, that's the beginning of the transsulfuration pathway). So we were interested in looking at children with Down syndrome to examine whether this pathway was altered. We did find alterations in the pathway.

We also looked at cells (the blastoid cell lines) from children with Down syndrome. What we see with Down's kids is that homocysteine is low because it is being pulled down the transsulfuration pathway with the overexpression of CBS. But, interestingly, we also found the glutathione levels were low, which you might not expect with overexpression of CBS. It turns out that superoxide dismutase (which leads to hydrogen peroxide) is also on 21 and that is overexpressed, and so we did see some oxidative stress in the children (the GSH-GSSG ratio being decreased), but (again) for a very different reason (secondary to another gene on chromosome 21).

We also were interested in the parents, and looked at the frequency of the MTHFR polymorphism (the 677T). This was actually a surprise. We just thought it might be interesting and it was a huge payoff. It was actually very controversial when our paper first came out.

Most geneticists involved with Down syndrome thought there really wasn't a genetic cause, but that it was somatic. What we found was a significant increase in the 677TT. The hypothesis (and I always have to have a mechanistic hypothesis to get interested in a project) was that basically Down syndrome is a nondisjunction event 95% of the time in the mother (two chromosomes don't separate properly). Thinking about that and the folate cycle was the rationale behind even thinking about looking at MTHFR. It turns out that where methyl groups are most concentrated is in the pericentromeric repeats (CG repeats) around the centromer. I hypothesized that if the mothers had a MTHFR polymorphism that should affect the methylation of the DNA, and where most methyl groups are is in this pericentromeric region.[11] With fewer methyl groups in the centromeric region, what happens is that the DNA is in a more open configuration and more likely to tangle and may, somehow, be involved (mechanistically) with why those chromosomes aren't segregating properly. That was the rationale.

We came out with this paper and it was very controversial. It usually takes 5 to 10 years before a hypothesis is replicated or not. The good news is that it is being replicated. And actually even more interesting, other genes that are involved in this pathway are now being found to be elevated (in MTRR and MTHR1298--other polymorphisms affecting the same pathway that would affect methylation). Data are coming out of different countries now that really seems to support that we were on the right track. Subsequent to that paper (our paper with this kind of far out hypothesis), came several papers showing that, in fact, the MTHFR677TT is clearly associated with DNA hypomethylation, which would fit with our hypothesis that maybe it is the methylation around the centromer that is involved with the nondisjunction event…

Let's go back to Laurette, where we started, because Laurette has just been instrumental in my life. This is a mother. Her child has Down syndrome, and developed leukemia. I got a telephone call out of the blue from this mother, who was obviously well read. She really had no biochemistry background, and it was fun because she couldn't really say the words right, but she knew what she was talking about. We formed a wonderful relationship, helping her child get through chemo, because children with Down syndrome are very sensitive to methotrexate. We formed this wonderful relationship, which has held today.

So through that Down's project, and through Laurette, we were doing a study (as I mentioned previously) looking at this pathway in children with Down syndrome. As researchers, one of the problems we have in doing human studies (in children, especially) is the availability of normal control children.

I had a great idea. The mothers of these Down's children were so helpful and so grateful. I asked them if they could bring in a normal sib. Usually this wouldn't be a good control, but for Trisomy 21, it is a fine control. And, of course, the moms were more than happy to bring in the group of kids, so we got a lot of controls.

One mom had twins, and one twin had Down's and the other twin had autism (no Down's, but just autism). That is what triggered my interest in Down syndrome. It was truly an N of 1, but it was so unusual. I couldn't tell which one was the control; I had to call to ask. It was usually very obvious which child was the control child. Based on that N of 1, and with Laurette's help, we decided to follow up. Was this was a fluke (something had been wrong) or was it real?

So it was truly serendipitous that I got to autism through Down's and through Laurette. She arranged a physician in Buffalo, and I said I needed 10 plasma samples from autistic children. Laurette, bless her heart (her daughter was somewhat autistic, as well as Down's, as well as getting through leukemia, so she's been through the gamut), had relations with the autism community there and arranged and got 10 plasma samples.

We repeated our profile and it was so consistent I really didn't believe it. I called the physician back (kind of embarrassed) and said, ‘You know, Paul, I'm not sure I believe this data because it is too consistent, and you don't

see that in humans, generally.' I asked him for another 10 plasma samples, and again (with Laurette's help), they sent me a second 10 samples. The results were the same (a little more variation, but basically the same pattern). That launched me, full speed, into autism and I haven't quit yet and don't intend to. That is how we got started."

What could this research mean for future approaches to autism? Dr. James:

"What we see--and this gives us so much insight into this imbalance in metabolism and what it might mean for etiology and for treatment targets--is a decrease in methionine levels, and that was highly statistically significant. In the paper, I break it into subgroups because the mean difference was significant (statistically), but within that mean (if you look at a subset), what we found is that half of those kids had extremely low methionine levels. That was kind of our approach. The mean differences were exactly as we had seen in the previously smaller set, but now that we have a larger group I can get more definitive because, as you know, autism is highly heterogeneous. We were really interested in what subset of kids was severely affected, and could we isolate that subset and then look at their behavior and try to make some sense of biomarkers versus behavior. What we found, again, was similar: decrease in methionine (very reproducibly), decrease in its product (S-adenosylmethionine) in about 50%, and then an increase in S-adenosylhomocysteine (the product/methylation inhibitor) in about 20%. Even though the mean difference was statistically significant, it was really a subset that I would consider functionally affected by the elevated SAH (which would affect their methylation). So then, of course, the ratio was decreased. We look at this ratio as the best indicator of methylation capacity because you have a low methyl donor (SAM) and (in a subset of the kids) a high SAH; that is a set-up for methylation problems. That was a new finding that we thought was very interesting.

If we look at the transsulfuration pathway, low cysteine is highly consistent. As I said, it is the precursor for glutathione synthesis. You would anticipate (with low cysteine) that you would have low glutathione, and we did see that as well. I think the most interesting (and the strongest) indicator that these children are under oxidative stress is the increase in plasma GSSG (that is the disulfide oxidized form of glutathione--it is the spent form that has given up its hydrogens and it is not being converted back to the active GSH as rapidly as it should be). The only reason that GSSG would be increased in the plasma is if there is a problem intracellularly. What the cell will do as a last-ditch effort when it can't keep up and that absolutely essential redox ratio begins to creep into a dangerous level is to get rid of that GSSG--export it, get it out of there, reduce the denominator--and keep that essential redox ratio in a good range. When we see an elevated GSSG in the plasma that is proof positive that there was a problem inside the cell, which is where we are really interested, mechanistically. That, I think, was our strongest indication that many of these children appear to be under chronic oxidative stress…

Basically what we have is a phenotype. When you look at the metabolic profile that gives you (in my mind) the sum total of the genes and the environment for that individual. It gives you clues about genetic susceptibilities. We have found (it was part of our most recent paper in the *American Journal of Medical Genetics*), several polymorphisms that are increased in autistic children that might be responsible for this abnormal profile.[12] The profile, itself, gives us clues possibly to etiology, as well. The problem with autism, intellectually, I think, for many physicians, is that it is a behavioral diagnosis and so you are thinking neuro; you are thinking brain. We are introducing a metabolic component, and that means it is going to affect systemically; it is going to affect beyond the brain because that pathway is in every single cell of the body. It brings out the possibility that if this is a genetic predisposition to this metabolic imbalance that is very environmentally sensitive, that maybe we are affecting more than the brain. In fact, maybe the brain is downstream…

I think all of this--our work and the work of others--is beginning to change the view of autism. It is much more than a neuropsychiatric condition, that there is systemic involvement. There are real medical problems--

gastroenterology and immunology--in these kids, and if we can treat them, they are going to get better. That is a whole other area that I think is so important in the transition to understanding to autism more as a medical condition rather than a neuropsychiatric disorder, and treating those medical problems with the children and some of them can get better. It's wonderful."

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