Solving the Mystery of Autism

FOR THE RESEARCH COMMUNITY FROM THE STATE UNIVERSITY OF THE HEALTH SCIENCES Winter 2004

THE UNIVERSITY OF MEDICINE & DENTISTRY OF NEW JERSEY

UMDNJ RESEARCH

Autism research: a cooperative effort

by **George Lambert**

GEORGE LAMBERT, MD, DIRECTOR, CENTER FOR CHILDHOOD NEUROTOXICOLOGY AND EXPOSURE ASSESSMENT; ASSOCIATE PROFESSOR, DIRECTOR, DIVISION OF PEDIATRIC PHARMACOLOGY AND TOXICOLOGY; UMDNJ-ROBERT WOOD JOHNSON MEDICAL SCHOOL

The recognition of autism as an escalating public health problem in the United States was demonstrated when the federal government conducted "The Autism Summit Conference: Developing a National Agenda" in Washington, DC, in November. Three major themes were addressed during the two-day event: biomedical research; services for individuals across the lifespan; and early screening and diagnosis. Also, a "10-Year Autism Research Roadmap" was unveiled.

Some of the credit for the federal government's growing awareness of autism goes to scientists in our own state, who participated in the CDC's Brick Township, NJ, study, published in June 2001, that documented an alarming prevalence of 6.7 cases of all autism spectrum disorders per 1,000 in the target population of children ages 3 to 10. This number was many times higher than was previously thought, and confirmed the concern of Brick Township parents who had raised the red flag. The much-publicized California study on autism, released in fall 2002, reported a doubling of newly diagnosed cases of full spectrum autism in that state during the four-year period from 1999 to 2002. Researchers are working to untangle what these figures actually mean: Are they indicative of an epidemic or are the growing public awareness of autism and changes in diagnostic criteria driving the numbers upward?

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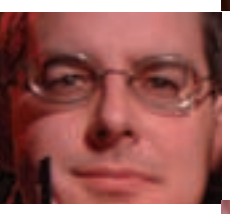
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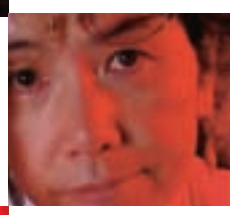
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The University of Medicine and Dentistry of New Jersey is a statewide network of eight schools on five campuses in Camden, New Brunswick/Piscataway, Newark, Scotch Plains and Stratford. The schools include New Jersey Medical School, Robert Wood Johnson Medical School, School of Osteopathic Medicine, New Jersey Dental School, Graduate School of Biomedical Sciences, School of Health Related Professions, School of Nursing and School of Public Health. The University has more than 4,500 students in 38 degree and certificate programs, 11,000 employees, including 2,075 faculty members, 17,000 alumni and more than 200 education and healthcare affiliates throughout New Jersey. The University is dedicated to pursuing excellence in the education of health professionals and scientists, conducting research, delivering healthcare, and serving the community. UMDNJ is ranked among the 100 top research universities in the country.

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From left to right: George Wagner, PhD, professor, Rutgers University; principal investigator, animal modeling project; George Lambert, MD, Department of Pediatrics, UMDNJ-Robert Wood Johnson Medical School (RWJMS); Center director, principal investigator, clinical sciences project; Kapila Seshadri, MD, Department of Pediatrics, RWJMS; Paul Lioy, PhD, Department of Environmental and Community Medicine, RWJMS, principal investigator, exposure assessment and intervention project; Paul Potito, executive director, NJ COSAC; Ken Reuhl, PhD, professor, Rutgers University, Center co-director, principal investigator, adhesion and repulsion project; and Audrey Mars, MD, Department of Pediatrics, RWJMS

Whatever the answer, the cost of autism to the child, family, community and nation is tremendous, particularly in the loss of human potential. But the financial burden is also huge: Our current estimate is that autism is costing the state of New Jersey more than one billion dollars per year. Clearly autism has become a public health problem that must be addressed.

New Jersey's current efforts are multi-focal. Earlier diagnosis is among the top priorities, since it has been demonstrated that intervention before age 3 can make a significant difference in the child's social and language development. Identifying the genes related to autism is an important goal, since genetic background seems to play a major role. Epidemiological studies will document the actual numbers, reveal clusters if they exist, and suggest new investigative avenues. If the incidence of the disorder is actually skyrocketing, then factors other than genes are at work. Scientists in the state are exploring the possible link to environmental chemicals, infections and autoimmune diseases. In addition, New Jersey researchers are furthering our knowledge of how to work better with autistic children to maximize their potential, and how to improve care of their multiple and complex medical problems.

Without a doubt, it will take the efforts of many researchers following different avenues – in this state and across the country – as well as clinicians, educators, family members and government leaders to grapple successfully with this baffling disorder. The University's scientists are in a strong position to make a difference for these individuals and their families, as you will see in this issue of *UMDNJ Research.*

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Investigating links between autism and the environment

by **George Lambert**

UMDNJ-Robert Wood Johnson Medical School's Center for Childhood Neurotoxicology and Exposure Assessment is one of only two such centers in the U.S. to study the interrelationships between autism and the environment. It has been funded for five years at more than $7 million by the National Institute of Environmental Health Sciences and the Environmental Protection Agency. The Center is conducting five interrelated and synergistic projects.

The Center was formed in response to a public health mandate for environmental studies in autism. While it is clear that genetics is a crucial factor, there are indications that the environment plays an important role in neurobehavioral development and the expression of autism. These indicators include an apparent increase in the incidence of autism over the last few decades, implying that other factors such as the environment are contributing to the expression of autism. Exposure to certain chemicals during pregnancy appears to be able to cause autism. Women who took the drugs thalidomide and valproic acid had an increased incidence of autism in their offspring. Since there are more than 160,000 chemicals in the environment, we can expect some of them to have a similar function or structure to thalidomide or valproic acid and perhaps contribute to the expression of autism.

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Children with autism exhibit behaviors that suggest they may be at greater risk from environmental chemicals and that these chemicals may play a role in autism itself. One of these behaviors – repeatedly putting objects and their own hands in their mouths – increases children's exposure to chemicals in and around their homes. Another associated observation is regression or loss of neurobehavioral function, which can occur in up to 30% of children with autism between 12 and 30 months of age. This period corresponds to the time when children begin to explore their environment, which increases their exposure to environmental chemicals. We are investigating a potential causal relationship between exposure to chemicals, regression and other autism associated behaviors.

Other concerns about autism and environmental chemicals come from studies of children without autism. Recent studies indicate that the average and accepted background levels of neurotoxicants such as lead in children without autism may be responsible for a loss of up to seven IQ points. Incredibly, no study has ever examined the effects of these background levels of neurotoxicants on children with autism, who may be even more susceptible, and whose development may be more profoundly altered by the resulting loss of neurobehavioral function.

Clearly there is a need for a comprehensive study of environmental factors in autism. The complexity of this endeavor dictates the involvement of basic and clinical scientists from multiple disciplines. The Center was formed in November 2001 with investigators from the medical school and Rutgers University who are working on two clinical and three basic science projects. These projects are theoretically and scientifically linked to examine similar questions with a unique but synergistic and cooperative approach.

The basic science projects have already begun to yield some important results. Dr. DiCicco-Bloom is examining environmental factors of societal concern, including methylmercury, lead and the teratogenic anticonvulsant, valproic acid, for effects on neurogenesis and differentiation. He has demonstrated in an animal model that very low levels of these neurotoxicants can have profound and regionally specific effects on brain growth in areas of the brain affected in children with autism. Dr. Reuhl has found that extremely low levels of some environmental chemicals can alter adhesion and repulsion molecules of the neurons that are partially responsible for how the brain is wired. Dr. Wagner has developed animal models of select behaviors found in autism, such as self-injurious behavior and regression. His preliminary data indicate that very small doses of certain chemicals administered before and after birth can induce self-injurious behavior and a regression-like effect in an animal model. Recently, his team has begun to explore the capacity of chemical antidotes to these neurotoxicant-related adverse effects.

One clinical project is investigating which environmental chemicals children with autism are exposed to. The second is comparing the children with autism who have regression to children with autism who do not have regression. The goals are to determine — in these two cohorts — which chemicals they are exposed to and whether there are differences in quantity of exposure to select chemicals and/or differences in regional brain growth patterns, genes associated with protection of the body from environmental chemicals or with speech, autoimmunity or development.

The blood, hair and urine levels of specific environmental chemicals of children in each cohort are measured. Blood is obtained from the mother, father, and child for identification of specific genetic polymorphisms as previously described. Researchers also assess the type and amount of environmental chemicals in the individual homes, and quantify the amount of mouthing of hands and objects by the child. In addition, global information systems (GIS) are used to determine the proximity of the house to known sources of environmental contaminants, such as underground storage tanks and manufacturing facilities. Neurodevelopmental and behavioral assessments of the children, as well as physical and neurological exams, are performed to determine many factors, including head circumference, how rapidly the skull is expanding and genetic analyses of the mother, father and child. Each child has a volumetric and functional MRI at least once during the study, and two years after the child enters the study, he will be behaviorally and environmentally reassessed.

This study benefits the child and family in several ways, which include a rapid and comprehensive pediatric, neurological, psychological and developmental assessment of the child, and a determination of the levels of environmental chemicals in the child and the home. If the home or the child has unacceptably high levels of environmental chemicals, clinical and/or environmental intervention strategies will be developed to reduce the exposure and risk, and follow-up studies will be done to assure resolution of the problem.

One hundred children between 24 and 36 months of age are being recruited for the study. After one year, 33 subjects and their families from New Jersey, New York, Pennsylvania, and Connecticut are enrolled, and 20 homes have undergone an initial evaluation. Preliminary results have revealed important information. As anticipated, children with autism place their hands, and objects such as toys, in their mouths far more frequently than children without autism. Preliminary functional MRI studies have revealed a remarkable change in the functional MRI from these children as compared to children without autism. In 20% of the homes, levels of environmental contaminants are high enough to warrant intervention.

The clinical projects mandate a community-based and cooperative approach. A multidisciplinary group of scientists leads such efforts with their support staff, including developmental pediatricians, neurogeneticists, GIS modelers, exposure assessors, psychologists and individuals conducting MRI and chemical analyses.

Equally important to the success of the Center and its projects is the participation of organizations from the autism community in New Jersey. Indispensable partners in the Center are The Community for Outreach and Services for the Autism Community (COSAC), the Eden Institute of Family Services in Princeton, and the Douglass Developmental Disabilities Center at Rutgers University, which are involved in protocol design, annual review of progress, and working with the greater autism community.

Future prospects for autism research look bright. Studies will lead not just to answers about autism, but also to a better understanding of normal brain development and function, as well as of other neurological dysfunctions and their relationship to the environment.

For more information about the Center and the clinical study or to volunteer for the clinical study, please call Dr. Lambert at 732-445-0174 or Stephanie Spencer at 732-445-0146.

George Lambert is an associate professor of pediatrics, director of the NIH/USEPA Center for Childhood Neurotoxicology and Exposure Assessment, and director of the Division of Pediatric Pharmacology and Toxicology at UMDNJ-Robert Wood Johnson Medical School. He earned his MD from the University of Illinois, and completed his residency at The Johns Hopkins Hospital and a research fellowship in molecular teratology at the National Institute of Child Health and Human Development.

Dr. Lambert – an internationally recognized expert on pediatric environmental health, pharmacology and toxicology – has conducted clinical studies in 10 countries. He serves on the Environmental Protection Agency's Science Advisory Board and the National Academy of Sciences committee on exposure. He is the P.I. and director of the NIH/USEPA Center, which is looking at the relationship between autism and environmental chemicals. Additional research includes the effects of in utero *exposure to environmental chemicals on adult male reproductive function. He also studies human development including neurobehavioral function, the incidence of adult diseases, liver function and birth defects, and the effects of occupational exposure to polycyclic aromatic hydrocarbons on liver and lung function and perinatal exposure to plasticizers. Widely published, his work has recently appeared in* JAMA, Lancet, Pediatrics, AJOG, *and* The New England Journal of Medicine.

EMANUEL DICICCO-BLOOM, MD, ASSOCIATE PROFESSOR OF NEUROSCIENCE AND CELL BIOLOGY, AND PEDIATRICS, UMDNJ-ROBERT WOOD JOHNSON MEDICAL SCHOOL

Understanding the development of the autistic brain

by **Emanuel DiCicco-Bloom**

It seems that everyone knows somebody affected by an autism spectrum disorder (ASD), leading some to think autism is increasing. While this remains controversial and difficult to establish retrospectively, enhanced public awareness and concern have brought increased private and national funding and more investigators to autism research. As a pediatric neurologist long interested in basic mechanisms of brain development, I had not originally considered this complex cognitive disorder within the domain of my research program. However, two experiences changed this. First, accumulating evidence based on neuro-imaging and pathology studies indicated that early brain development is significantly altered in autism. Second, several studies of autistic children identified changes in developmental regulators, specifically growth factors, a number of which we have studied for years. Consequently, our work is highly relevant to understanding autism pathogenesis. In turn, it has been very exciting and rewarding that our studies of developmental and environmental factors are helping address the challenges faced by those affected by autism. Through collaboration among developmental neuroscientists and human geneticists, new insights into autism causation are now emerging that will lead to earlier diagnosis and more effective therapies over the next decade.

Childhood presents wonderful and challenging experiences for families. The young child engages his parents with broad smiles and twinkling eyes to share excitement over new-found discoveries, speaks sounds and words to obtain food and playthings, and actively explores the world without concern of danger. But social engagement, spoken language and unfettered

THROUGH COLLABORATION AMONG DEVELOPMENTAL NEUROSCIENTISTS AND HUMAN GENETICISTS, NEW INSIGHTS INTO AUTISM CAUSATION ARE NOW EMERGING THAT WILL LEAD TO EARLIER DIAGNOSIS AND MORE EFFECTIVE THERAPIES OVER THE NEXT DECADE.

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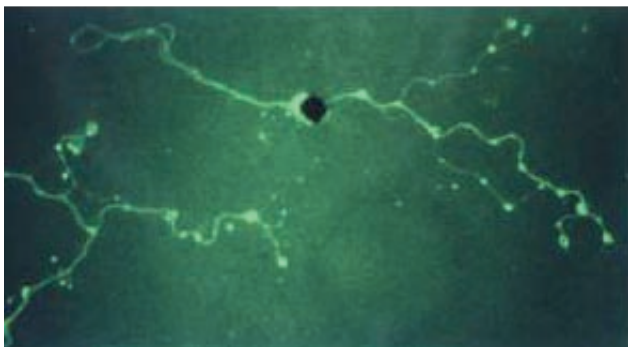
exploration in those with autism are impaired or absent, leading to abnormal interpersonal relationships. These core deficits occur in combinations unique to each individual, making this spectrum disorder difficult to define and study. Research from around the world indicates that ASD occurs in 1 to 2 children per 500. While abundant evidence indicates this is primarily a polygenic disorder, likely involving 3 to 20 genes, environmental factors of maternal or community origin may also contribute. Consequently, autism in specific individuals is probably caused by different groups of genetic and environmental factors, potentially explaining the marked heterogeneity of signs and symptoms.

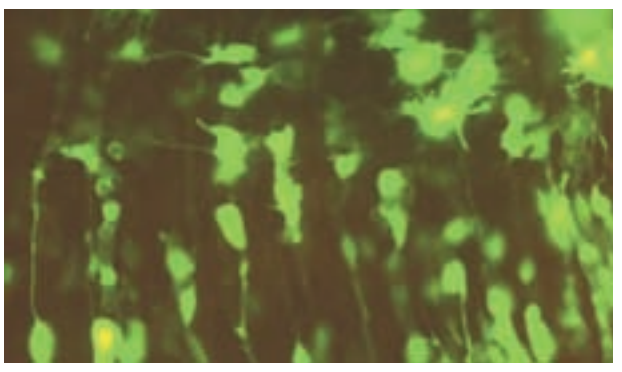
The inherent variability in behavioral and cognitive models was a major force in my choice to study basic mechanisms of brain development, starting with undergraduate research on frog embryos. My initial studies focused on individual neurons, which I examined under the microscope, taking movies as single precursors divided in two, a rare image in the neuroscience world. Using these primary cell culture models, I spent much time identifying protein growth factors and peptides, which direct cells to divide or cease dividing, or promote survival and differentiation. However, as a pediatric neurologist seeing children with abnormal brain development, I knew well the clinical importance of showing that molecules we characterized in culture also served as functional regulators in developing animals, which are useful models of humans.

Little did I suspect that my interests would be redirected by attending an Eden Institute Autism Conference in 1996 at Princeton University, where Dr. Marie Bristol-Powers of the National Institute of Child Health and Human Development (NICHD) spoke about a seminal work, *The State of the Science of Autism: A View from the NIH*. Discussions that day introduced me to autism as a disease of human brain development, and culminated in an invitation to serve on the Scientific Advisory Board of the National Alliance for Autism Research (NAAR), a newly created advocacy group with the goal of funding basic research on causes of autism. While delighted to review research proposals on basic brain development and consider relationships to autism, I was fairly certain I would not study the disorder. Two important events changed my views. Soon after joining NAAR, "Dateline NBC" televised a report of apparently remarkable improvements in language and bowel function after a child with autism received a secretin challenge test, where secretions from the pancreas are measured following intravenous injection of this normal gut peptide. While there was great skepticism in the medical community, many families called physicians to obtain secretin for their children. Since secretin is a member of a peptide family which I study, including Vasoactive Intestinal Peptide (VIP) and Pituitary Adenylate Cyclase Activating Polypeptide (PACAP), the Director of NICHD asked me to speculate on whether secretin could in fact affect brain function in children with autism. *In vitro*, we had found that VIP and PACAP could stimulate neural precursor cell division, enhance survival, and stimulate differentiation, whereas in living animals, peptides and growth factors crossed the blood brain barrier to elicit developmental effects. While there was no evidence of secretin receptors in relevant brain regions nor of rapid and direct secretin effects in the brain, NICHD was compelled to conduct several nationwide studies on secretin effects in autism, finding no supportive evidence.



Top: Two embryonic rat neurons are shown with highly branched processes and oval cell bodies, one of which has a black nuclear label indicating it is engaged in the mitotic cycle. Wolf et al, 1996.

Bottom: This section of embryonic rat cerebral cortex shows neuronal precursors in the proliferative zone that were genetically altered in vivo*, indicated by expression of green fluorescent protein. Following Caesarean section, DNA vectors were injected directly into the embryo's cerebral ventricles by passing through the maternal uterine wall.*



Next came the report by Dr. Karin Nelson, leading pediatric neurologist and epidemiologist at NIH, and Dr. Judy Grether in California, indicating that growth regulators important in brain development, including brain derived neurotrophic factor (BDNF), the related NT4, VIP and CGRP were elevated in the blood of newborns who later developed autism. While this remarkable and convincing finding has yet to be replicated, I was again invited to the NIH (NINDS) to address the issues, and became convinced that our studies of VIP and BDNF in developing brain were relevant to ASD. Indeed, recent evidence shows markedly elevated levels of BDNF in autopsy samples of autistic brains.

A closer look at the neuro-imaging and pathology indicated that autistic brains show many abnormalities that could occur only before birth, during the earliest stages of nervous system formation. The abnormalities are diverse and non-uniform, but on the whole, represent disturbances in the entire sequence of development, including neuronal cell production (neurogenesis), survival, migration, differentiation and neuronal process elaboration and regression. Remarkably, despite the primacy of social, communication and cognitive impairments, functions that reflect activity of the more recently evolved cerebral cortex in the forebrain, abnormalities of the hindbrain, closer to the spinal cord, were also important. This was an unexpected finding, since psychologists and neurologists find no obvious related clinical signs. For example, autism occurred frequently in children exposed to the anti-nausea drug thalidomide taken by their mothers during the first month of gestation, when the neural tube is closing and hindbrain neurogenesis predominates. At this time, VIP/PACAP and BDNF regulate several developmental processes. Furthermore, the most consistent pathological abnormality is a deficiency of

Purkinje neurons in the cerebellum, a major hindbrain structure, whereas neighboring cranial neurons and fiber pathways are also disturbed. Finally, in neuro-imaging studies of children by Dr. Eric Courchesne, hindbrain and forebrain growth is abnormal, showing rapid acceleration during the first years of life. We have modeled excessive brain growth and increased neurogenesis in the newborn rat, using a single injection of a critical growth regulator, basic fibroblast growth factor (bFGF), and are examining in parallel the impact of environmental factors.

Our current autism work focuses on three overlapping strategies. First, we continue defining the effects of and mechanisms by which VIP/PACAP, BDNF and bFGF regulate the numbers and kinds of neurons generated in autism-related forebrain structures, the cerebral cortex and hippocampus, and the cerebellum in the hindbrain. These studies identify intracellular pathways and cell cycle machinery mediating developmental responses, examined by exposing precursors in culture to growth factors or DNA vectors and by injecting them into embryos *in utero* or into newborn pups. Developmental changes seen in autism may reflect abnormal control of the timing or activities of these regulatory pathways. Second, we are examining environmental factors of societal concern, including methylmercury, lead and a teratogenic anticonvulsant associated with disease, valproic acid, for effects on neurogenesis and differentiation, to address possible disease contributions. Finally, we are collaborating with mouse geneticist James Millonig and human geneticist/psychiatrist Linda Brzustowicz (see articles on pages 7 and 9) to determine whether molecular regulators we identify as important for brain development in animals are associated with the human disease.

Specifically, growth factor systems and transcription factors that control them are tested using DNA samples from autistic individuals and their families to see whether genetic changes are over-represented in the affected children. This approach has recently borne fruit: A transcription factor, ENGRAILED2, that patterns the formation of neurons in the hindbrain, and especially cerebellar Purkinje neurons, is mutated in autism, as recently reported by Millonig and Brzustowicz. This finding underscores the value of a developmental animal model approach to defining human ASD, a strategy that is already identifying additional genes. Through these collaborations, we expect to uncover a diversity of interacting genes and signals involved in the autism spectrum, while we prepare the next generation of physician-scientists to focus on this common yet poorly understood disorder.

Emanuel DiCicco-Bloom graduated summa cum laude from Princeton University, and received his MD from Cornell University Medical College, where a National Research Service Award supported his postdoctoral studies on growth factors in neural development with Ira B. Black, MD. Following pediatric and neurology training at New York Hospital-Cornell Medical Center, a Clinical Investigator Award supported his pioneering research on growth factor regulation of cell division in primary neuronal precursors. Dr. DiCicco-Bloom came to UMDNJ-Robert Wood Johnson Medical School in 1990 to the departments of Neuroscience and Cell Biology and Pediatrics, and has been supported by NINDS, NICHD, NIEHS/EPA, FDA, NAAR and CBTF. He is a principal investigator at the Center for Childhood Neurotoxicology and Exposure Assessment. He serves on the Scientific Advisory Board and the Medical Affairs Committee of NAAR, and has reviewed for NIH study sections and the NJ Governor's Council on Autism. Dr. DiCicco-Bloom is on editorial boards of many neuroscience journals and is associate editor of the International Journal of Developmental Neuroscience, and guest editor of the journal's special issue on Autism.

Unraveling the genetics of autism

by **Linda Brzustowicz**

Autism is a serious disorder at least partially caused by genetic factors. One of the clinical features of autism is abnormal language development. We have previously studied the genetics of a form of language delay that has some features in common with the language problems of autism, and have identified the chromosome location of a gene that is involved in the language delay. Interestingly, this location has separately been implicated in the etiology of autism. We are now starting a new genetic study that will recruit families with one individual with autism and additional family members with a history of language problems, to directly test if we can identify gene variants that are involved both in autism and non-autism language problems.

Autism is a complex disorder of unknown etiology in most cases. While environmental factors likely play a role in the illness, it is also clear that certain individuals inherit a genetically determined susceptibility to developing autism. Over the past 10 years, medical science has made remarkable strides in identifying the specific genetic defects that lead to many human diseases. However, most of these diseases are quite unlike autism, being caused by a defect in a single gene, often with no or limited contribution from environmental factors. Recent estimates suggest that alterations in as many as 10 or 20 different genes may be involved in the inherited component of autism.

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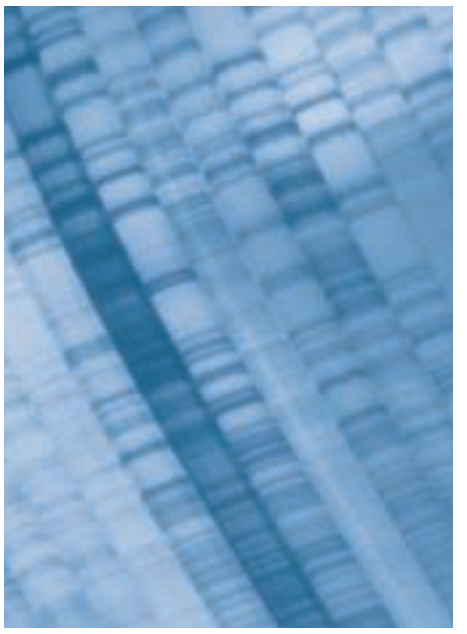

Our laboratory uses techniques of modern molecular genetics to try to better understand what makes some people more susceptible to certain behavioral disorders, including autism. Our studies typically begin with the recruitment of families with more than one individual affected by a particular illness. We then collect blood samples from all interested family participants, using this as a source to extract DNA from each individual. Then, in our laboratory, we compare the DNA sequences from the different family members to see if we can identify any specific regions of particular chromosomes that all the people with the illness have in common. In this way, we hope to identify genetic variations related to the

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LINDA BRZUSTOWICZ, MD, ASSOCIATE PROFESSOR, DEPARTMENT OF GENETICS, RUTGERS UNIVERSITY; ASSOCIATE PROFESSOR, DEPARTMENT OF PSYCHIATRY, NEW JERSEY MEDICAL SCHOOL

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illness. The key feature of this type of study is that it requires families with multiple individuals affected with the disorder.

While there has been much news of late about the increase in the rate of diagnosis of autism, it is still a relatively rare disorder, and it is difficult to find families with multiple individuals affected with autism. For a disorder as genetically complicated as autism, one would ideally like to have at least several hundred families with more than one person with autism available for study. Collecting such a large group of families is difficult, and nationwide recruitment efforts have been needed to obtain such samples in the past.

Our laboratory came to study autism through a somewhat unusual route. Together with Dr. Paula Tallal's group at Rutgers-Newark, we have been studying the genetics of a disorder called specific language impairment, or SLI, for the past 10 years. SLI is clinically defined as failure to develop language normally in the absence of any known explanatory reason such as hearing loss, mental retardation, oral motor abnormalities, or neurological or psychiatric disorder. SLI is not a rare disorder, estimated to affect 7% of school-age children. In 2002, our group published the results of a genetic study of SLI in a set of extended families from Canada. We found evidence that there was a gene located on chromosome 13 that conferred significant risk for developing SLI in these families. While fascinating in its own right, we were also very excited to find that this exact region of chromosome 13 had been implicated in susceptibility to autism.

Autism is characterized by abnormalities in three areas, the development of language, social responsiveness, and repetitive or rigid behaviors. Although the language abnormalities of autism are typically more severe than those of SLI, some studies have suggested that the pattern of deficits is similar in quality, if not severity. In addition, studies of relatives of individuals with autism have reported increased rates of language difficulties, although these family members had typically not been assessed with the level of detail necessary to make a diagnosis of SLI. Even the autism study implicating chromosome 13 found that the evidence for this gene was strongest in those families where the children with autism exhibited significant delays in the onset of multi-word speech and when parents who reported a history of language problems were also considered as affected individuals.

All of these findings prompted us to design a new study that would directly test the hypothesis that there may be a genetic overlap between autism and SLI. The underlying premise is simple. If the genetic component of autism is caused by variations in multiple interacting genes, then it is possible that relatives who inherit a certain subset of these variants may express something similar to the language problems of autism, but not as

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severely. Since language problems are much more common than autism, particularly among the relatives of individuals with autism, it should be relatively easy to identify families with one individual with autism but multiple other individuals with a history of language problems. Instead of needing to scour the entire country to find sufficient families with multiple individuals affected with autism, we should be able to find sufficient families for our study right here in New Jersey. And if the language problems in these families are caused by a subset of the variations needed to cause autism, the task of finding these genes should be easier, as we will be trying to identify fewer interacting genes.

The National Institute of Mental Health recently funded our proposal for this study, and we plan to begin the process of family recruitment shortly. Dr. Charles Cartwright, acting director of the Autism Center at New Jersey Medical School, will head the clinical assessment portion of the project, with my laboratory conducting the genetic analyses. While our study design is most likely to identify only a certain number of the genes involved in autism (namely the ones contributing most to the language problems), this will help in the overall understanding of autism. The unraveling of the genetics of a complex disorder like autism is like solving a jigsaw puzzle; each piece that can be assembled, no matter how small, makes the placement of the next piece that much easier. As a complement to our family-based genetic studies, we are also collaborating with Drs. James Millonig and Emanuel DiCicco-Bloom, both of Robert Wood Johnson Medical School, to test the possible involvement in humans with autism of candidate genes that they have identified through their work in animal model systems. We plan to pursue as many parallel avenues of research as are feasible to accelerate our understanding of this serious disease.

Linda Brzustowicz attended Harvard University as an undergraduate and earned her MD from Columbia University College of Physicians and Surgeons. She completed a residency in psychiatry at the Columbia University Department of Psychiatry/New York State Psychiatric Institute, and spent three additional years at Columbia as a research fellow, training in molecular and statistical genetics. She has held joint appointments at Rutgers and UMDNJ since 1994, and currently runs the Psychiatric Genetics Laboratory in the Rutgers University department of genetics, focusing on the study of the genetics of autism and schizophrenia.

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ENGRAILED2 links genetics and cerebellar development to autism

by **James Millonig**

Autism is a devastating behavioral disorder that affects the development of the central nervous system (CNS). Sixty years ago, Leo Kanner described the first autistic disorder. Years of research have changed our understanding of the disease and its development, which has led to new ideas about its causes. Autism spectrum disorder (ASD) has three clinical diagnoses: autism, Asperger's syndrome, and Pervasive Developmental Delay-Not Otherwise Specified (PDD-NOS). Autism is characterized by severe communication impairments, social interaction deficits, and repetitive/stereotypic behaviors. Asperger's syndrome is distinguished from autism by a less severe language impairment. Individuals affected by PDD-NOS fit some but not all the criteria necessary for an autism diagnosis. ASD afflicts approximately 6.7 per 1,000 individuals in the general population, with four males affected to every one female.

Three lines of evidence support the idea that ASD is a polygenic disorder. First, monozygotic twins have a 75 to 90% concordance rate for ASD compared to only a 10% concordance rate for dizygotic twins. Second, families that have a child diagnosed with ASD have about a 5 to 8% chance of having another child affected with ASD. Third, linkage analysis has identified numerous chromosomal regions that segregate with ASD. Disease modeling studies suggest that between five and 15 genes interact with each other and the environment to cause ASD.

The CNS structure most commonly affected in autistic individuals is the cerebellum. Twenty-one out of 22 autopsy studies display cerebellar abnormalities, including a decrease in the number of Purkinje cells. Cerebellar hypoplasia has also been reported in autistic individuals. These defects occur in the absence of any obvious sign of degeneration, suggesting that autism is caused by developmental defects. Recently, the growth pattern of the cerebellum during childhood has also been shown to be abnormal. Initially, cerebellar growth is accelerated in autistic individuals compared to unaffected controls but then declines after age 6. The cerebellum is traditionally thought to control just motor coordination. However, functional MRI studies have recently demonstrated that the cerebellum is active during activities that are deficient in ASD, including language generation, attention and problem solving. Together these experiments demonstrate that cerebellar development is disturbed in autistic individuals and that these defects might contribute to the behavioral abnormalities observed in ASD.

Scientists working on mouse genetics have identified a number of genes that function during cerebellar development. One such gene is *Engrailed2* (*En2*), a homeobox transcription factor that is orthologous to *Drosophila melanogaster engrailed*. Both loss of function and transgenic misexpression mutants have been generated for the mouse *En2* gene. Interestingly, both types of mutations display a phenotype that is reminiscent of the cerebellar anatomical abnormalities reported for autistic individuals. Adult mice for each single mutant are non-ataxic but their cerebella are hypoplastic with a reduction in the number of Purkinje cells and other cell types. Closer anatomical examination of these mice has revealed that these phenotypes are due to abnormal post-natal development. In addition, human *ENGRAILED2* (*EN2*) maps to a region of the genome, 7q36.3, which has displayed suggestive linkage to ASD in three separate studies from two independent genome scans.

For these reasons, *EN2* was examined as a susceptibility locus for ASD by performing family based association analysis. Family based association studies investigate whether certain alleles of a gene are inherited more frequently in affected than in unaffected siblings. If a single nucleotide polymorphism (SNP) is not linked to a mutation associated with ASD, then both alleles of the SNP will be transmitted to affected offspring 50% of the time. However, if a SNP is linked to a mutation, then one of the alleles should be over-transmitted (>50% transmission) to affected

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JAMES MILLONIG, PHD, ASSISTANT PROFESSOR, FACULTY MEMBER, DEPARTMENT OF NEUROSCIENCE AND CELL BIOLOGY, UMDNJ-ROBERT WOOD JOHNSON MEDICAL SCHOOL AND MEMBER, CENTER FOR ADVANCED BIOTECHNOLOGY AND MEDICINE; RYM BENAYED, GRADUATE STUDENT, ROBERT WOOD JOHNSON MEDICAL SCHOOL

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siblings but under-transmitted to unaffected siblings. The extent to which the transmission of the alleles deviates from the expected 50:50 distribution within a large population determines the statistical significance of the association.

To test whether human *EN2* contributes to ASD, I entered into a collaboration with Linda Brzustowicz, MD, who is an expert in polygenic neuropsychiatric disorders, including autism (see page 7). My graduate student, Rym Benayed, and Neda Gharani, PhD, an assistant research professor in Dr. Brzustowicz's lab, worked closely to test whether SNPs found within the *EN2* gene were over-transmitted to ASD-affected individuals. Families recruited to the Autism Genetic Resource Exchange (AGRE) were used for these studies. AGRE is a central repository of family DNA samples created by The Cure Autism Now (CAN) Foundation and the Human Biological Data Interchange. The selection criteria require that at least two family members have a diagnosis of autism, Asperger's syndrome or PDD-NOS. These DNAs are distributed by the Rutgers University Cell Repository headed by Jay Tischfield, PhD, chair of the Department of Genetics at Rutgers University.

EN2 is encoded by two exons separated by a single intron in 8.0kb of genomic DNA. SNPs from every 1.0kb were analyzed for transmission distortions from parents to affected and unaffected offspring. We discovered that two SNPs, *rs1861972* and *rs1861973*, located 156 bp apart in the intron, are significantly over-transmitted in ASD-affected individuals. The *rs1861972* SNP is an A/G polymorphism with the A nucleotide being the common allele, while the *rs1861973* SNP is a C/T polymorphism with the C nucleotide being the common allele. Initially, the transmission of these SNPs from parents to an autistic child was analyzed in 135 triads (parents and one autistic child from each pedigree). Significant over-transmission of the *rs1861972* A-allele to affected offspring was observed (P=0.0018). A similar distortion of transmissions was observed for the *rs1861973* SNP (P=0.0003). Next, the inheritance of both SNPs together as a haplotype was analyzed. The *rs1861972* and *rs1861973* A-C haplotype is specifically over-transmitted to affected individuals (P=0.00011). The transmission of these SNPs was then investigated in all affected and unaffected siblings in 167 AGRE pedigrees. Significant over-transmission to affected but not unaffected children was again observed under both a narrow (autism) and broad (autism, Asperger's or PDD-NOS) diagnostic criteria (narrow: *rs1861972*

WE DISCOVERED THAT TWO SNPs, *rs1861972* AND *rs1861973*, LOCATED 156 bp APART IN THE INTRON, ARE SIGNIFICANTLY OVER-TRANSMITTED IN ASD-AFFECTED INDIVIDUALS.

P=0.0290; rs*1861973* *P*=0.0073; haplotype *P*=0.0009; broad: *rs1861972* *P*=0.0175; *rs1861973* *P*=0.0107; haplotype *P*=0.0024). A similar analysis was performed for other SNPs located in the intron or exons of *EN2* but they demonstrated no significant association. Consistent with this finding, the *rs1861972* and *rs1861973* alleles segregate with each other ($P=6.4 \times 10^{-49}$) but not with the flanking non-associated SNPs. Other data indicate that the *rs1861972* and *rs1861973* SNPs are not the causative mutations but instead are tightly linked to the functional variant.

In summary, these data demonstrate the significant association of a cerebellar patterning gene with ASD, suggesting a role for *EN2* as a susceptibility locus and supporting the hypothesis that genetic alterations that affect cerebellar development could predispose individuals to both autism and related ASD. Cis-regulatory sequences are often located within the first intron of genes so it is possible that the functional variant associated with ASD causes the misregulation of EN2 during cerebellar development. During mouse cerebellar development, the spatial and temporal regulation of *En2* is tightly regulated. For example, from embryonic day 17.5 to post-natal day 4 (P4), *En2* is expressed in spatially restricted "stripes." Within these "stripes," *En2* is expressed in all primary cerebellar cell types (granule, deep nuclei and Purkinje cells). However, by P4 a developmental switch occurs so that *En2* is no longer expressed in Purkinje or deep nuclei cells, but is now restricted to differentiating granule cells. In an *En2* transgenic mouse in which expression of *En2* in Purkinje cells is prolonged past P4, adult mice exhibit an "autistic-like" cerebellar phenotype, a decreased number of Purkinje cells and hypoplasia. Interestingly, the *En2* knockout mouse also displays hypoplasia and a reduced number of Purkinje cells, indicating that decreased levels of *En2* could result in a similar phenotype. Both mutations disrupt the topographic mapping of spino-cerebellar mossy fibers, which could in turn affect the electrophysiological function of the cerebellum. Together these data suggest that functional variants in human *EN2* that affect either the levels or the spatial/temporal expression of the gene during human cerebellar development might contribute to the anatomical cerebellar phenotypes observed in autism and in turn play a role in the underlying etiology of autism. Future work will focus on identifying the functional variant within *EN2* and determining whether the variant disrupts the levels and/or expression of *EN2*. Continued analysis of *EN2* and the pathways regulated by *En2* during mouse cerebellar development will provide further insight into the genetic and developmental basis of ASD.

James Millonig graduated magna cum laude from the University of Rochester with a BS in Biochemistry. In 1988, he completed his Master's degree at Oxford University in England in prokaryotic molecular genetics. He earned a PhD in 1994 in molecular biology from Princeton University, performing his doctoral research in mouse genetics and transcriptional regulation. He then joined Mary E. Hatten's lab at Rockefeller University, where he applied his mouse genetic background to study the development of the cerebellum. In 1999 he became an assistant professor in the Center for Advanced Biotechnology and Medicine and a faculty member in the Department of Neuroscience and Cell Biology at Robert Wood Johnson Medical School. Dr. Millonig is also an adjunct assistant professor in the Department of Genetics at Rutgers University.

Adverse reactions to dietary proteins in children with autism

by **Harumi Jyonouchi**

Children with autism spectrum disorders (ASD) often suffer from various medical conditions. Treating these conditions could improve their cognitive development. Among the most common medical problems found in children are gastrointestinal (GI) symptoms, including chronic diarrhea, unformed stool, bloating, constipation, GI cramping, gastroesophageal reflux and ulcers. Parents often report that intake of certain foods seems to be associated with these GI symptoms. Many dietary intervention measures have been promoted for improving cognitive development in ASD children without solid scientific proof. However, the gluten-free, casein-free (gf/cf) diet appears to have consistent, favorable clinical responses that have been observed by many physicians. Our initial results indicate that in ASD children there is evidence of significant cellular immune reactivity to common dietary proteins, especially cow's milk protein. This may partly explain the favorable results of such dietary interventions. Our current studies focus on the underlying mechanisms of such aberrant immune reactions causing reactivity to dietary proteins. We are also identifying objective markers for ASD children who are most likely to respond to the dietary intervention measures.

Autism spectrum disorders (ASD) is an umbrella term for a group of developmental disorders diagnosed on the basis of behavioral phenomena. However, clinical features of ASD are more likely the result of expression of various etiological and pathological factors. They likely vary at different stages of development, leading to markedly variable behavioral manifestations and degrees of cognitive impairment. Without definitive therapeutic measures, children with ASD require considerable medical and social resources for their care and education.

In addition to behavioral symptoms, ASD children frequently exhibit medical problems that appear to be associated with immune abnormalities. These include frequent and prolonged illnesses with common microbes and unusual adverse reactions to benign environmental factors such as dietary proteins (DP). A series of studies also indicate increased familial autoimmunity in ASD children. In a subset of ASD children, parents have described associations between changes in behavioral symptoms and common childhood illnesses and the intake of certain foods. However, it is unclear how such underlying medical problems affect behavioral symptoms and cognitive development in ASD children.

Nevertheless, a variety of dietary supplements and intervention measures have been promoted as "boosting" immune functions, improving cognitive activity and attenuating aberrant behaviors in ASD children. These measures usually fall under the heading of alternative and complementary medicine. Since there is no cure and no standard medical treatment for ASD, these measures are likely to attract desperate parents' attention, although their efficacy, in general, has not been tested with rigorous scientific measures. A gluten-free, casein-free diet appears to be very popular. Many parents report improvement of GI symptoms, autistic behaviors and cognitive activity, indicating this dietary intervention likely

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has some clinical benefit in a subset of ASD children.

ASD children often manifest gastrointestinal (GI) symptoms starting from infancy but prior to the onset of autistic behaviors and developmental delay. GI inflammation, characterized by lymphoid nodular hyperplasia and prominent epithelial cell damage (so-called "autism colitis") has been reported in children with regressive autism. Although the pathogenesis of "autism colitis" is unclear, many parents report an association between intake of certain foods (most commonly dairy and wheat products) and exacerbation of GI symptoms and autistic behaviors. Parents of such ASD children believe their children suffer from a "food allergy," but a conventional allergy work-up to detect IgE-mediated immune responses is negative in most of these ASD children.

The gut mucosal immune system in infants is immature and less tolerant of foreign antigens, including common dietary proteins (DPs). As a result, infants often develop immune responses against common DPs, most frequently milk and soy proteins. These immune responses are seldom associated with IgE antibodies (Abs) that cause immediate immune responses. Instead, cell-mediated immunity appears to play a vital role in infants with an adverse reaction to dietary proteins (DP intolerance: DPI). Many infants outgrow DPI in parallel to maturation of the gut immune system and establishment of tolerance in the gut mucosa. However, some infants take a prolonged period to establish immunological tolerance to DPs, developing persistent GI inflammation.

Our previous results indicate that ASD children may have a high prevalence of DPI mediated by cellular immune reactivity. This may be partly associated with aberrant innate immune abnormalities found in ASD children. Although most ASD children eventually outgrow DPI with age, they appear to take longer than typical children to do so. This may be partly due to their poor communication skills and inadequate use of language, hence the delay of implementation of appropriate avoidance measures of causative DPs. Delay of the DPI diagnosis may make ASD children more vulnerable to persistent GI inflammation and possibly aggravate autistic behaviors and cognitive impairment.

A VARIETY OF DIETARY SUPPLEMENTS AND INTERVENTION MEASURES HAVE BEEN PROMOTED AS "BOOSTING" IMMUNE FUNCTIONS, IMPROVING COGNITIVE ACTIVITY AND ATTENUATING ABERRANT BEHAVIORS IN ASD CHILDREN.

We thus postulate that cellular immune reactivity to DPs and resultant GI inflammation can partly explain the favorable effects of the gf/cf diet in ASD children. However, previous studies evaluating the effects of the gf/cf diet produced inconclusive results. These results might be associated with random assignment of ASD children to the elimination diet irrespective of GI symp-

toms and age of study subjects. For example, older ASD children are more likely to have established immunological tolerance spontaneously, as in normally developing children, and may not show improvement of behavioral symptoms and cognitive functions on a restricted diet. Many ASD children have skewed eating habits and sensory disintegration issues that make implementation of the elimination diet quite challenging and stressful for the entire family.

Because the gf diet is especially difficult to implement, parents are often discouraged by pediatricians from trying it. This is because normally developing children outgrow DPI at an early age. Also, pediatricians have concerns for the patient's nutritional status, since ASD children often already have self-restricting diets. Given the fact that the most common causative DPs for adverse immune reactions in infants are cow's milk protein (CMP) and soy protein, if the ASD child is only reactive to CMP, the implementation of the cf diet could be sufficient for a good clinical outcome. Unfortunately, there are no commercially available laboratory tests to identify potential responders to dietary intervention measures or causative DPs inducing adverse immune reactions.

To address the role of immune abnormalities and adverse reaction to DPs in clinical features of ASD and the effects of the gf/cf diet, we are conducting cross-sectional and prospective clinical studies. In the cross-sectional studies, we have been evaluating cellular immune reactivity to common DPs in comparison with its reactivity to other recall antigens, lipopolysaccharide (a surrogate stimulant for innate immunity), and polyclonal T cell stimulants (mitogens) in ASD children with or without GI symptoms [GI (+) or GI (-)]. In prospective studies, we also attempt to assess other confounding factors that influence the effects of dietary intervention measures and assess improvement of GI and behavioral symptoms after implementation of the appropriate diet.

Our initial results in the cross-sectional study indicate that TNF-alpha production against CMP along with IL-12 is frequently seen in GI (+) ASD children and even in some GI (-) ASD children when they are on a regular diet. Moreover, these values appear to decrease in ASD children who have been placed on the gf/cf diet for more than six months. Most parents report improvement in behavioral symptoms (especially speech and hyperactivity), along with resolution of GI symptoms following implementation of the elimination diet imposed on the basis of immune reactivity detected in our laboratory. However, such assessment was conducted over a short follow-up period (two to three months) and in some cases such favorable effects are lost as a consequence of other insults such as viral gastroenteritis, rhinosinusitis, fungal overgrowth, etc. It remains to be seen how such measures alter cognitive development in ASD children in the long run.

Our future research efforts will also focus on underlying mechanisms of such immune dysfunction. In this way, we hope to elucidate a link between immune abnormalities and resultant clinical conditions, such as DPI, behavioral symptoms and cognitive impairment in ASD children. We are in the process of expanding our studies to address these issues.

These studies were supported by The Jonty Foundation, St. Paul, Minnesota. This private, nonprofit organization promotes funding for biomedical research in ASD.

Harumi Jyonouchi is an associate professor in the Division of Pulmonary, Allergy, Immunology and Infectious Diseases, Department of Pediatrics, UMDNJ-New Jersey Medical School. She was recruited from the University of Minnesota in July 2002. Dr. Jyonouchi is board certified in pediatrics and allergy/immunology and her research projects focus on patients with ASD, chronic rhinosinusitis, and other immunodeficiency disorders in relation to innate immune abnormalities and subsequent aberrant adaptive immune responses.

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Autism, brain growth and stem cells

by **Richard S. Nowakowski and Nancy L. Hayes**

One consistently identified difference between individuals with autism and those without is the size of the brain at birth and the amount and rate of change in brain size in the first few months after birth. In our studies of the developmental mechanisms which regulate brain size, we have found that the proliferating stem cells make the right number of neurons by "minding their P's and Q's."

One of the great mysteries of autism and autism spectrum disorders is that although the symptoms are remarkably similar from patient to patient and the diagnosis is often clear, it is by no means apparent what parts of the brain are affected in these disorders. Many laboratories have addressed this issue using magnetic resonance imaging (MRI). The results from these studies consistently indicate that the size of the brain of autistic patients differs from age-matched controls. Moreover, in the few months just before and just after birth, the size of the brain of children who later will be diagnosed as autistic changes more and faster than the brain size of children who will not receive this diagnosis.

In order to understand how brain size may be related to autism, we have been studying the mechanisms that control the normal development of the brain by examining the kinetics of the cell cycle of stem cell populations. We are looking at the behavior of the proliferating stem/progenitor cells of the brain, both in embryos and in the adult hippocampus, one of the parts of the brain in which stem cells persist throughout adulthood. Why the cell cycle? The cell cycle is the sequence of events that a cell goes through when it duplicates itself, producing two "daughter" cells. Thus, with each pass through the cell cycle, the number of proliferating cells doubles. If the cell cycle is 8 hours long, then within 24 hours the number of proliferating cells will first double, double again, and then double a third time. In other words, one cell will turn into 8 cells in only one day. If the cell cycle is shorter, for example, 6 hours, then in the same 24 hours, one cell will turn into 16 cells; whereas if it is longer, for example, 12 hours, then one cell will become only 4 cells. Thus, the change in the length of the cell cycle is a powerful mechanism by which differences (increases or decreases) in the size of the brain could occur.

In the developing neocortex of mice, we have shown that neurons are produced from the 11th day of gestation through the 17th day of gestation. During this six-day period, the length of the cell cycle of the stem/progenitor cells increases from about 8 hours to more than 18 hours. This means that, in princi-

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ple, during these 6 days the productive capacity of these stem/progenitor cells decreases and the neocortex is built with 11 passes through the cell cycle by the stem/progenitor cells. In human development, a similar but much longer sequence of events occurs during the mid-gestational period, during which the cortex is built by ~35 cell cycles over three to four months.

If all of the daughter cells produced during this neurogenetic interval re-entered the cell cycle, then for each stem/progenitor cell present at the beginning of the period in the mouse a total of 2^{11} or 2,048 cells would be produced. This tremendous expansion of the stem/progenitor population does not occur, however, because a second control step enters into play. The second control step is a regulation of the proportion of daughter cells that re-enter the cell cycle (P) vs the proportion of daughter cells that quit the proliferative cycle to become neurons (Q). Note that because P and Q are proportions, their sum is equal to 1. Changes in P and Q are just as influential on cell production as changes in the length of the cell cycle. For example, if P is 1 (and Q is 0), then the stem/progenitor population will double with each cell cycle as described above. However, if P is less than 1, then the stem/progenitor population will not double but some "Q" cells will be produced that can then differentiate into neurons. During the 11 cell cycles that are needed to make the mouse neocortex, we have shown that P and Q change systematically. At first P is slightly less then 1 and Q is slightly greater than 1, so the proliferative population expands greatly and produces a few neurons. Then, around cell cycle 7, P falls below 0.5 and Q rises above 0.5, so the proliferative population starts to contract, but even more neurons are produced per cell cycle. By cell cycle 11, P becomes 0 and Q becomes 1, and the stem/progenitor population "disappears" because both daughter cells of each proliferating cell exit the cell cycle.

TO GAIN INSIGHT INTO THE POSSIBLE GENETIC INFLUENCES ON THESE IMPORTANT VARIABLES, WE HAVE BEGUN TO STUDY THE VARIATION IN PROLIFERATION OF THE STEM/PROGENITOR CELLS AND NEUROGENESIS IN THE ADULT HIPPOCAMPUS OF NUMEROUS INBRED STRAINS OF MICE.

With this basic understanding of the normal sequence of events of the stem/progenitor cells in the neocortex, we hypothesize that changes in either or both cell cycle length and the rate of change in P and Q affect the size of the brain in autism. To gain insight into the possible genetic influences on these important variables, we have begun to study the variation in proliferation of the stem/progenitor cells and neurogenesis in the adult hippocampus of numerous inbred strains of mice. Differences in the brains among these

strains in many ways parallel differences found in the brains of individual humans. However, because a given inbred strain of mice is genetically homogeneous, we can examine many specimens with identical genetic make-up. We have found that the properties of stem/progenitor cells in the adult hippocampus vary in different inbred strains. There are genetic controls on the number of stem/progenitor cells, the differentiation and survival of newly produced cells as they integrate into the circuitry of the brain and their distribution within the hippocampus. Interestingly, we have not yet identified any differences in the length of the cell cycle for these cells.

The next steps in our research will be to identify the genes that control stem/progenitor cell behavior during adult neurogenesis and to determine if any of these are also involved in regulating the embryonic development of the brain. Then, we will have a set of candidate genes that other researchers (see article by James Millonig, p. 9) will be able to use to evaluate whether or not they play a role in the developmental bases of autism or whether or not the biochemical pathways involved are affected by environmental changes.

Nancy L. Hayes and Richard S. Nowakowski are both associate professors in the Department of Neuroscience and Cell Biology at UMDNJ-Robert Wood Johnson Medical School. Dr. Hayes received her PhD in Neurobiology from the University of North Carolina. Dr. Nowakowski received his PhD in Cell and Developmental Biology from Harvard University. This husband and wife team works together on issues related to cell proliferation in the developing brain and during adult neurogenesis.

Does oxidative stress play a role in autism pathogenesis?

by **Sue X. Ming**

Although the etiology of autism remains elusive, it is thought that most cases are the result of an interaction between genetic and environmental factors. A causal mechanism has been identified for a small percentage of those affected with this developmental disorder. One of the better understood causes of autism is exposure to thalidomide during pregnancy. Thalidomide causes fetal malformation in rabbits by a mechanism involving a process called oxidative stress. Another agent, valproic acid (VPA), is also associated with autism if the fetus is exposed during pregnancy. Valproic acid causes behavioral and biochemical alterations similar to the developmental deficits and biochemical alterations observed in human autism.Valproic acid causes oxidative stress in humans and possibly in a mouse model of autism. This article describes our interest in investigating a possible role of oxidative stress in autism pathogenesis.

The interaction of genetic and environmental factors thought to cause autism is expressed during fetal and early child development. Many laboratories are trying to identify these factors in order to understand autism disease processes (pathogenesis). We were inspired by the report that thalidomide use during early pregnancy was highly associated with autism in a small cohort. In the early 1960s, thalidomide was prescribed for pregnancy-related morning sickness, but its use was banned worldwide when it was found to cause limb malformation in the developing fetus. Now, decades later, it becomes clear that thalidomide causes more than limb malformation. In addition, an unusually large percentage of those exposed to thalidomide early in pregnancy also developed autism. We speculate that the underlying mechanism for limb malformation and autism may be similar.

The mechanism responsible for this fetal malformation was shown to be mediated by the production of free oxygen radicals (super oxygen species), as indicated by elevated tissue levels of a biomarker of oxidative stress, 8-hydroxy-2-deoxyguanosine (8OHdG). In animal studies, when the free radicals were neutralized by prior treatment with a free radical trapping agent, the fetal malformation was prevented. Thus we speculated that free radicals might participate in thalidomide teratogenesis in humans and contribute to some features of autism. However, two thirds of human fetuses exposed to thalidomide during the critical period did not develop autism, suggesting that other factors were involved, such as a genetic predisposition that might exacerbate or fail to protect against oxidative stress.

Another agent, the anticonvulsant valproic acid, has also been associated with increased incidence of autism if administered to mothers during pregnancy. This agent, like thalidomide, is known to cause oxidative stress. Valproic acid therapy has been demonstrated to increase a free radical biochemical process called lipid peroxidation and to decrease the activity of the antioxidant enzyme, glutathione peroxidase (GPX). Valproic acid is associated with abnormal fetal neurodevelopment in rats in a manner consistent with the pathology of human autism and thalidomide teratogenesis. These lines of evidence suggest that oxidative stress may contribute to valproic acid toxicity. Although we now realize that thalidomide and valproic acid both cause oxidative stress and have been associated with autism, most cases of autism are unrelated to either agent. However, many other environmental toxicants have been shown to cause oxidative stress, including heavy metals, insecticides, infection or even food additives. In addition to environmental factors causing oxidative stress, another mechanism of oxidative stress is reduced antioxidant capacity. Antioxidants counteract the free radicals to limit the damaging effects of oxidative stress. These antioxidant defense mechanisms can be altered by many factors. Of particular importance are the genetic variants (polymorphisms) of the antioxidant enzymes such as GPX. Diminished antioxidant defense mechanisms would exacerbate oxidative stress, especially when the organisms are challenged. Thus, individuals with reduced antioxidant capacity may be predisposed to disease processes such as amyotrophic lateral sclerosis (Lou Gehrig's disease). One small scale study of antioxidants in autism found that children with autism have reduced plasma levels of two antioxidant enzymes, catalase and GPX.

ALTHOUGH WE NOW REALIZE THAT THALIDOMIDE AND VALPROIC ACID BOTH CAUSE OXIDATIVE STRESS AND HAVE BEEN ASSOCIATED WITH AUTISM, MOST CASES OF AUTISM ARE UNRELATED TO EITHER AGENT.

In our studies we found that children with autism have a significant increase in excretion of one of the biomarkers of oxidative stress, isoprostane.

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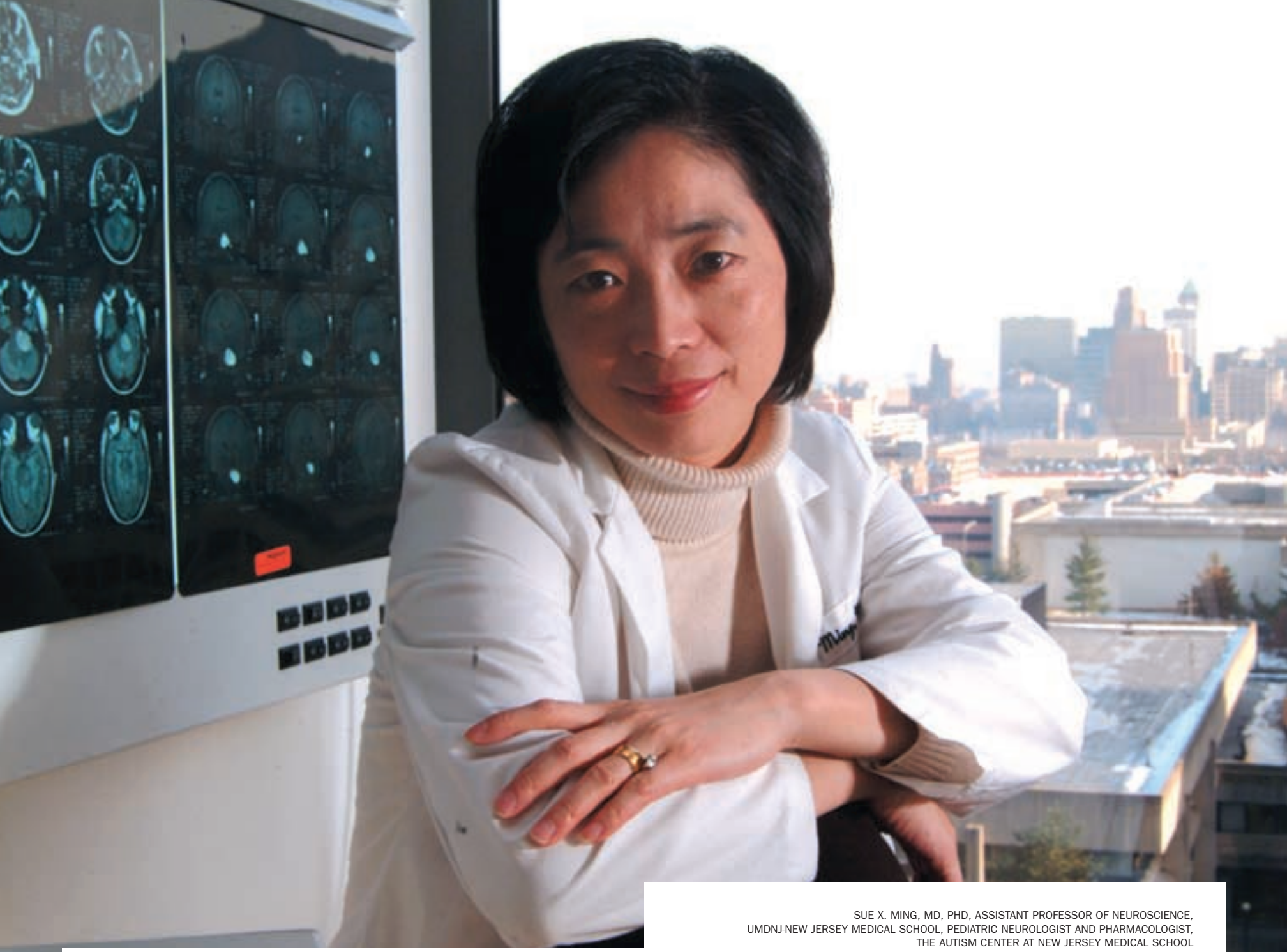

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There was an average four-fold increase in urinary isoprostane levels in 33 children with autism as compared with 29 age-matched healthy control children. There was also a slight trend of increased excretion of 8OHdG in those children with autism. We are currently confirming and extending these preliminary results. We are also planning to measure the antioxidant capacity in these two groups of children and to examine the genetic polymorphism of antioxidant enzymes in children with autism and their families. This study is being conducted in collaboration with Dr. Peter Stein at the UMDNJ-School of Osteopathic Medicine and Drs. William Johnson and George Lambert at UMDNJ-Robert Wood Johnson Medical School.

In addition to these studies on human subjects, we are also investigating whether oxidative stress plays a role in an animal model of autism. Dr. George Wagner at Rutgers University has developed a mouse model of autism in which mice are treated with VPA during pregnancy or shortly after birth. These mice exhibit retardation in the acquisition of motor skills, deficits in learning, regression of motor skills, and self- injurious and aggressive behaviors. These effects are accompanied by an increase of a neurotransmitter, serotonin, in brain regions such as the striatum and hippocampus, as well as alterations in the gene expression of neural cell adhesion molecules (molecules involved in brain development) 24 hours after VPA treatment. A preliminary study of oxidative stress in the brains of VPA-treated mice revealed an increase of isoprostane levels as compared with the levels in saline-treated mice. The behavioral retardation, regression and self-injurious and aggressive behaviors, as well as the biochemical alterations, are consistent with the developmental deficits and the biochemical alterations found in human autism. Thus, VPA-treated mice may be an important animal model of autism.

Working with Drs. Wagner and Alycia Halladay, also from Rutgers, we demonstrated that the behavioral deficits of VPA-treated mice were abolished when the mice were treated with the antioxidant vitamin E. This suggests that oxidative stress may be involved in the VPA-induced behavioral deficits in mice and that vitamin E may be effective in preventing behavioral deficits. We are currently examining other antioxidants for this ability to prevent VPA toxicity.

A PRELIMINARY STUDY OF OXIDATIVE STRESS IN THE BRAINS OF VPA-TREATED MICE REVEALED AN INCREASE OF ISOPROSTANE LEVELS AS COMPARED WITH THE LEVELS IN SALINE-TREATED MICE.

Sue X. Ming is a pediatric neurologist and pharmacologist at The Autism Center at New Jersey Medical School (NJMS) and an assistant professor of neuroscience. She received her MD from Shanghai Medical School in China and her PhD in pharmacology from NJMS. She completed her pediatric residency at SUNY-Health Science Center at Syracuse, NY and pediatric neurology fellowship at Johns Hopkins Medical School. She has been at NJMS for more than six years.

Determining the prevalence of autism

by **Walter Zahorodny**

Numerous recent reports from educational and health service providers across the United States point to the possibility that the number of children with autism may be increasing. Data from the New Jersey Department of Education disclose a large increase in the number of children with autism over the last decade. While the education service data can help us identify trends, this information cannot help us determine autism prevalence. The available data suggesting an increase in autism may, in fact, only reflect changes in the identification or classification of this disorder. To establish the prevalence of autism in our state, the New Jersey Autism Study is carrying out a population-based, multiple source, autism surveillance study. Determining autism prevalence in New Jersey will lead to the implementation of an autism monitoring system in the state and to reliable detection of changes in autism frequency or expression in the future.

Autism is a developmental disorder characterized by impairment of social interaction and understanding, communication deficits, and the expression of behavioral anomalies like compulsions, stereotypes and unusual play. Learning and attention problems, as well as difficulty modulating arousal, complicate the presentation of autism in many children and call for intensive educational remediation. The deficits associated with autism persist across the lifespan. Caring for the child with autism demands much from affected families and takes a personal and emotional toll on family members. Beyond the implications for an individual with autism and the family, there are significant consequences for the community and the country. Special education for a child with autism in New Jersey may cost more than $50,000 per year and families and public agencies bear the responsibility of paying for needed interventions. The more people affected with autism, the more resources that are needed, such as teachers, diagnostic professionals and treatment providers, and the greater the financial demand on already-strained educational and health service budgets.

Over the past decade, we have witnessed a rise in concern about the growing number of children diagnosed with autism. This concern goes hand-in-hand with increases in the number of children with an autism spectrum disorder (ASD) classified for special education. Data from the New Jersey Department of Education, for example, show an increase in autism-classified children from 446 to 2,925 during the period 1992 to 2002. This 559% increase is consistent with a similar (544%) increase in national levels of children with autism served by the Individuals with Disabilities Education Act (IDEA) during the period.

Apparent increases in a condition like autism lead to questions about whether the estimated number of affected people is accurate and, if so, what is accounting for the rising prevalence of this disorder. Unfortunately, there were no ready sources of reliable data about this until recently. In the early 1990s, Frank Desposito, MD, James Oleske, MD, and I started seeing greater numbers of autistic children in our practices. We started searching for ways to offer integrated care to these children. The conventional view, mainly based on European studies, was that autism was a rare condition, occurring 4 or 5 times per 10,000 children. Available U.S. data on autism prevalence was questionable. Only three studies of autism prevalence were conducted in the U.S. since 1998. These studies reported autism prevalence rates from 2.5 to 4.0 per 10,000 in the areas studied (Arkansas, North Dakota and Utah). Since these studies were carried out with limited access to health and educational records, they did nothing to address our concerns or clarify our questions.

There are several reasons why the number of people counted as having a disorder changes. One reason is a change in the way a condition is identified and classified; another is an increase or decrease in the actual occurrence, that is, changes in the prevalence or incidence of a condition. Another reason is an increase in the number of people at risk for the condition, such as when birth rates go up. In the case of autism, major re-definitions of autism occurred during the past 15 years. Revisions of the Diagnostic and Statistical Manual of Mental Disorders (DSM) of the American Psychiatric Association formulated in 1987 (DSM-III-R) and 1994 (DSM-IV), respectively, introduced a broader category of autism called pervasive developmental disorder not otherwise specified (PDD-NOS). This disorder shares many features of autism, without having all the behaviors necessary for a full autism diagnosis. The DSM also confirmed an autism variant, Asperger's syndrome, in which the affected person acquires language, but has significant social impairment. These changes in the classification/definition of autism made determination of changes in autism prevalence more difficult.

BECAUSE MORE PEOPLE ARE AFFECTED WITH AUTISM, MORE RESOURCES ARE NEEDED, SUCH AS TEACHERS, DIAGNOSTIC PROFESSIONALS AND TREATMENT PROVIDERS, AND THE FINANCIAL DEMAND ON ALREADY-STRAINED EDUCATIONAL AND HEALTH SERVICE BUDGETS IS GREATER.

Epidemiology allows us to compare how often a condition arises in one population as compared with another, to compare the frequency of the condition at different points in time and to understand the risk factors and causes of a disorder. Though rigorous epidemiologic methods have often been used to monitor the frequency and expression of communicable diseases, they have only infrequently been applied to developmental disorders, like autism.

In 1998, the Centers for Disease Control and Prevention (CDC), responding to concerns of a possible autism cluster, implemented a population-based autism prevalence investigation in Brick Township, NJ. The children studied in this community were 3 to 10 years of age and were Brick residents in 1998. The Brick Township investigation consisted of case identification and verification of possible cases from multiple sources and the administration of a "gold standard" diagnostic test for autism. The prevalence of Autistic Disorder was determined by this study to be 40 per 10,000 children, while the prevalence of autism spectrum disorders (Autistic Disorder, PDD, Asperger's) was 67 per 10,000 children. The prevalence estimate of autism in Brick Township determined by this investigation was significantly higher than previously published autism prevalence rates and called for a broader, epidemiologically rigorous investigation of autism in New Jersey.

Motivated by the Brick Township findings, Dr. Desposito and I developed

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WALTER ZAHORODNY, PHD, ASSISTANT PROFESSOR, DEPARTMENT OF PEDIATRICS, UMDNJ-NEW JERSEY MEDICAL SCHOOL; AND FRANKLIN DESPOSITO, MD, PROFESSOR, PEDIATRICS, AND DIRECTOR, CENTER FOR HUMAN AND MOLECULAR GENETICS, UMDNJ-NEW JERSEY MEDICAL SCHOOL

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a plan for determining autism prevalence in four New Jersey counties (Essex, Hudson, Union, Ocean), a region encompassing an annual birth cohort of 35,000. Our goals were to establish a reliable estimate of ASD in our region and to create the system for monitoring changes in autism prevalence. Our autism surveillance plan, the New Jersey Autism Study (NJAS), secured collaborative support from the CDC and necessary permissions from the New Jersey Department of Education, clinicians and autism diagnosis and treatment centers to implement a multiple-source, population-based autism prevalence study. At this time, NJAS data collection is ongoing. NJAS activities are now focused on children born in 1992. Children suspected of having autism are identified through careful screening and abstraction of records at numerous clinical sources and at the 82 school districts in the study area. Review and abstraction is implemented by specially trained NJAS staff working in accordance with specific standards. Determination of autism case status is made by developmental specialists reviewing all abstracted information using the DSM-IV autism criteria for Autistic Disorder, PDD-NOS and Asperger's syndrome. NJAS autism case ascertainment activities will be completed in June, at which time the study team will turn to analyzing the dataset, calculating prevalence rates, comparing rates based on sex, race, place of residence and other demographic factors. Since NJAS is abstracting information about the prescription of psychoactive medicines, it will also be able to offer population-based estimates of the frequency with which autistic children are treated with drugs.

Autism is not a rare disorder, as once thought. Activities of the New Jersey Autism Study will soon determine baseline autism prevalence rates for a large, demographically complex region. The initial autism prevalence estimates determined by our investigation will be a starting point for longitudinal monitoring of changes in the frequency and expression of autism in New Jersey, but will also be referenced to findings of autism researchers using similar methods in other states. To determine possible causes of autism and whether some children are at greater risk for autism spectrum disorders, our research team is now working to develop and implement case control studies comparing risk factors, such as family health history and environmental exposures, in children with and without autism.

ACTIVITIES OF THE NEW JERSEY AUTISM STUDY WILL SOON DETERMINE BASELINE AUTISM PREVALENCE RATES FOR A LARGE, DEMOGRAPHICALLY COMPLEX REGION.

Walter Zahorodny is an assistant professor in the Department of Pediatrics at UMDNJ-New Jersey Medical School. He received his PhD in clinical psychology from the New School for Social Research in 1988, and completed his clinical internship at New Jersey Medical School. He was an International Research and Exchanges Board (IREX) Developmental Fellow. Dr. Zahorodny's research interests include the epidemiology of autism and auditory processing of autistic children. He and Franklin Desposito, MD, are principal investigators of the CDC-supported New Jersey Autism Study, which will establish autism prevalence in New Jersey, as well identify patterns, trends, subgroups and risk factors of individuals with autism.

Secondary psychiatric diagnoses in patients with autism spectrum disorder

by **Marianne Tracey and Christine Schroeder**

This article examines the relative frequency of secondary psychiatric diagnoses in patients with autism spectrum disorders. These frequencies are compared to corresponding information for patients without autism spectrum disorders. Variations in the frequency and type of secondary psychiatric diagnosis across different types of autism spectrum disorders are also described.

Our practice serves clients with developmental disabilities, including autism spectrum disorders, and has maintained a database of client information since 1988. This database includes background demographic and medical variables as well as the diagnoses and treatment recommendations that result from a patient's initial consult. Many of the individuals, both with and without autism spectrum disorders, have one or more additional Axis I and Axis II psychiatric diagnoses.

The purpose of this study was threefold:

- To characterize the most common secondary psychiatric diagnoses in patients with autism spectrum disorders;
- To compare these psychiatric diagnoses to the diagnoses of patients without an autism spectrum disorder;
- To compare the relative frequencies of psychiatric diagnoses among patients with different types of autism spectrum disorders.

Provisional diagnoses ("rule in/rule out") were not included in the data, and the report does not include individuals with provisional autism spectrum disorder diagnoses.

Because we are assessing an existing patient group rather than a randomly selected group of individuals, these results are not assumed to generalize to the corresponding populations. The results are therefore presented in a descriptive format and we did not use inferential statistics.

Incidence of psychiatric disorders

This section compares the relative frequency of secondary psychiatric diagnoses in patients with and without autism spectrum disorders, and includes two groups:

- Patients diagnosed with an autism spectrum disorder and at least one secondary Axis I or Axis II psychiatric disorder (n=390);
- Patients with no autism spectrum disorder diagnosis with at least one psychiatric disorder (n=1420).

Table one presents the five most common psychiatric diagnoses for individuals with and without an autism spectrum disorder diagnosis. It was possible for patients to have more than one diagnosis.

Several things are notable from comparison of these two tables:

- Bipolar disorder had a much higher relative frequency in patients with an autism spectrum disorder (35% versus 12%). Similar, though less divergent, results were present for anxiety disorders (24% versus 15%).
- In contrast, organically based psychiatric disorders were less frequently diagnosed in patients with autism spectrum disorder (17% versus 27%), as was psychosis (12% versus 4%).
- Although personality disorders were the second most common diagnosis in patients without an autism spectrum disorder (16%), they were quite rare in patients with an autism spectrum disorder; the relative frequency of

Table one: Most common psychiatric diagnoses given at initial consult

Autism spectrum disorder	*No Autism spectrum disorder*
Bipolar disorder (35%)	Organically based disorder (27%)
Anxiety/OCD (24%)	Personality disorder (16%)
Organically based disorder (17%)	Anxiety/OCD (15%)
Depression (7%)	Depression (14%)
Intermittent explosive disorder (5%)	Bipolar disorder (12%)
Psychosis (4%)	Psychosis (12%)
ADHD (3%)	Intermittent explosive disorder (10%)

ANDREW LEVITAS, MD, MEDICAL DIRECTOR, THE DIVISION FOR PREVENTION AND TREATMENT OF DEVELOPMENTAL DISORDERS, UMDNJ-SCHOOL OF OSTEOPATHIC MEDICINE (SOM); CHRISTINE SCHROEDER, PHD, DATABASE DESIGNER AND STATISTICAL ANALYST, THE DIVISION FOR PREVENTION AND TREATMENT OF DEVELOPMENTAL DISORDERS, SOM; NOT PICTURED: MARIANNE TRACEY, EDD, DIRECTOR, THE DIVISION FOR PREVENTION AND TREATMENT OF DEVELOPMENTAL DISORDERS, SOM

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personality disorders in patients with an autism spectrum disorder was less than 1%.

Thus, while patients with an autism spectrum disorder were much more likely to be diagnosed with bipolar and anxiety disorders, they were less likely to be diagnosed with an organically based psychiatric disorder, psychosis, or a personality disorder.

Patterns of psychiatric disorder for different types of autism spectrum disorder

Patients with autism spectrum disorders can present with a wide range of symptoms and severities. Therefore, we also examined the above-presented information organized by autism spectrum disorder diagnosis.

This section includes two groups:

- Patients diagnosed with an autism spectrum disorder and at least one secondary Axis I or Axis II psychiatric disorder (n=390).
- Patients diagnosed with an autism spectrum disorder and, other than mental retardation, no secondary Axis I or Axis II psychiatric disorders (n=654).

Table two gives frequency of each type of autism spectrum disorder for these two groups combined.

Due to the small number of patients in the "other related disorders" category, only patients diagnosed with autism, PDD-NOS, or Asperger's disorder were included in the following statistics.

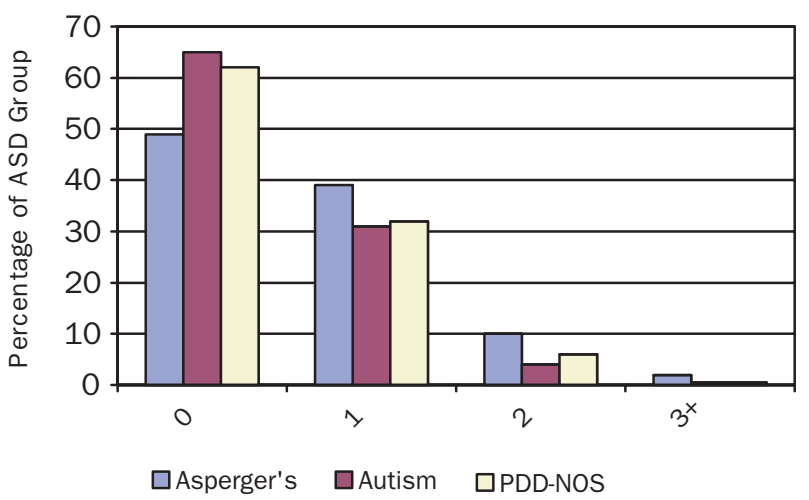
Chart one presents the number of secondary psychiatric diagnoses for individuals, grouped by autism spectrum disorder type. The number of diagnoses ranged from zero (those with no additional psychiatric diagnoses) to three or more.

Table two: Frequencies of autism spectrum disorder types

Asperger's disorder	51
Autism	472
PDD-NOS	516
Other related disorders	5

These results indicate that patients with Asperger's disorder were somewhat more likely to have additional psychiatric diagnoses. It is possible that patients with poorer levels of function may be more difficult to diagnose with psychiatric disorders, and patients with Asperger's disorder are generally quite high functioning (see Chart two).

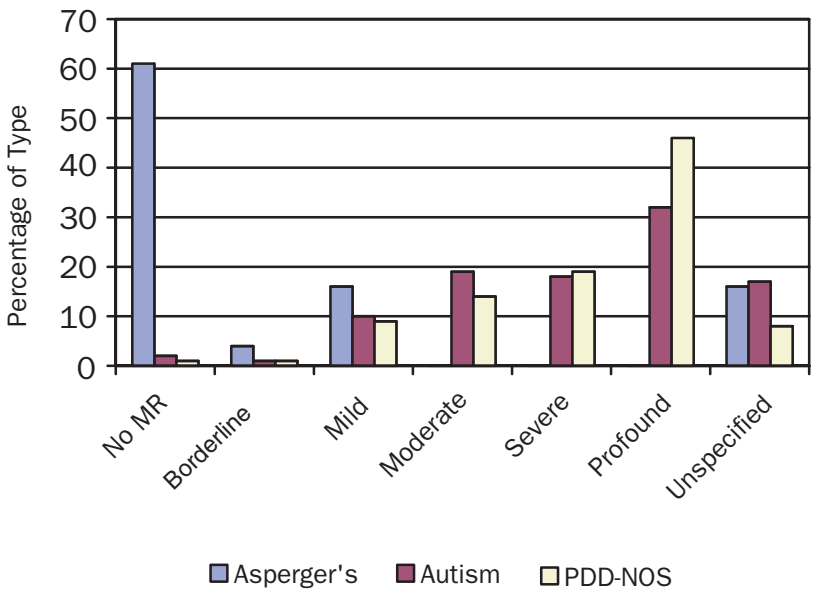
Chart one: Number of additional psychiatric diagnoses for each ASD type



Nonetheless, these results emphasize that, despite their high to near-normal levels of function, individuals with Asperger's disorder were the most likely of individuals with autism spectrum disorder to be diagnosed with at least one secondary psychiatric disorder.

Table three gives the most frequent psychiatric diagnoses for individuals with each type of autism spectrum disorder diagnosis.

Chart two: Level of function for each ASD diagnosis



These results indicate that secondary psychiatric diagnoses tend to be similar across autism spectrum disorder types. Patients with Asperger's disorder, however, tend to be diagnosed more frequently.

Table three: Most common psychiatric diagnoses by autism spectrum disorder type

Asperger's	*Autism*	*PDD-NOS*
Anxiety/OCD (18%)	Bipolar disorder (15%)	Bipolar disorder (10%)
Bipolar disorder (12%)	Anxiety/OCD (8%)	Anxiety/OCD (9%)
Depression (6%)	Organically based (4%)	Organically based (9%)
Tics/Tourette's (6%)	Depression (3%)	Depression (3%)
Organically based (4%)	Intermittent explosive disorder (2%)	Psychosis (2%)

Conclusions

Our patients with autism spectrum disorders were more likely to be diagnosed with bipolar and anxiety disorders than patients without autism spectrum disorder diagnoses. They were less likely to be diagnosed with organically based disorders, psychosis, or personality disorders. Among patients with autism spectrum disorders, patients with Asperger's disorder were the most likely to have additional psychiatric diagnoses.

Marianne Tracey is director of the Division for Prevention and Treatment of Developmental Disorders at UMDNJ-School of Osteopathic Medicine. She is responsible for developing and administering the services of the division, which cares for the psychological and behavioral needs of individuals with developmental disabilities. Christine Schroeder is a database designer and statistical analyst for the Division for Prevention and Treatment of Developmental Disorders.

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MICHAEL BRIMACOMBE, PHD, ASSOCIATE PROFESSOR, DEPARTMENT OF PREVENTIVE MEDICINE, UMDNJ-NEW JERSEY MEDICAL SCHOOL AND UMDNJ-SCHOOL OF PUBLIC HEALTH

Epidemiology, bioinformatics and autism: the search for patterns

by **Michael Brimacombe**

Autism is a neurological disorder that seems to be caused by unknown genetic and environmental factors. My research in autism stems from my interest in public health and the prevention of birth defects. In an era of genetics and genomic information, the goal of preventing many childhood disorders is a real possibility. Finding the specific causes of autism, as well as identifying its clinical subtypes, will require sifting through evidence from many different medical research areas. This can be done by conducting focused epidemiologic and clinical studies, as well as by examining larger databases of genomic and neurologic data.

As a biostatistician and epidemiologist, the epidemiologic and informatic aspects of autism research are my focus. I am participating in several ongoing autism research projects, ranging from narrowly focused clinical examinations of specific autistic symptoms and co-morbidities to a large-scale assessment of autism prevalence. My work involves both the planning of studies and interventions and the statistical and mathematical modeling of results. I am currently in the process of designing epidemiologic case-control studies to examine potential causes and related medical issues in autistic children.

Since autism is an unexplained illness with causes that are potentially both genetic and environmental, it is important to begin identifying clinical subtypes. This approach will allow for more detailed investigation of potential risk factors including *in vitro* fertilization, genetic status and specific co-morbidities such as prenatal difficulties and birth history related problems. This research, which employs case-control and cohort studies, is being carried out in collaboration with clinicians in the Autism Center at NJMS.

Initial results have shown that, compared to national averages, mothers giving birth to autistic children have significantly higher levels of premature delivery, vaginal bleeding, prolonged labor, Caesarean delivery, multiple births and diabetes. Often they have more than one of these difficulties. This agrees with previous findings. Furthermore, medical histories of family members of children in the cohort show high levels of developmental, psychiatric and medical problems. These findings support the view that autism is a systemic disorder. As understanding of the genetics underlying autism continues to develop, these clinical subtypes may provide useful guidance in determining levels of risk and treatment.

I have also helped guide the development of the Autism Brain Tissue Data Portal, located at the Autism Tissue Project, National Alliance for Autism Research. This neuroinformatic database is a collection of data derived from neurological examinations of donated brain tissue from autistic subjects. This resource will link various research areas providing a new type of research environment for neurologic researchers, enabling us to

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investigate broader, interdisciplinary neurologic hypotheses, and link to the various types of neurologic data such as fMRI visual images, clinical data and genomic microarray data. This work is at the level of basic research, examining the structures of the brain and identifying differences in autistic brains. It is part of the new field of bioinformatics.

The prevalence of autism in the U.S. is an issue provoking much debate. I serve as epidemiologist for the New Jersey portion of the Centers for Disease Control and Prevention Autism Prevalence Study, which is being conducted in four New Jersey counties. This is a large, collaborative epidemiologic study requiring standardized collection procedures for the abstraction and processing of data and assessment of results.

While my research is aimed at identifying the causes of autism, the issue of how to best manage and treat children with autism is an important one. The burden of care for individuals with autism falls primarily on the family. As the various levels of severity of autism become better understood, the need to assess family caregiver burden in relation to health outcomes will grow in importance. The development of approaches to assess this burden is also one of my research interests and will be the focus of studies we carry out involving cohorts of autistic patients at UMDNJ.

Separating the underlying causes of autism in order to understand the roles of genetics and specific exposures is a real challenge. The design and development of epidemiologic case-control studies to identify and describe clinical subtypes of autism will aid in the development of treatments for the condition and its symptoms.

Michael Brimacombe received his PhD at the University of Toronto and has worked at New Jersey Medical School and the School of Public Health for the past four years. He currently teaches biostatistics in the graduate program at the School of Public Health. His research is funded by the Centers for Disease Control and Prevention.

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Another view of autism

by **James Oleske**

Despite the lack of epidemiological studies confirming an association between autism and the MMR (measles/mumps/rubella) vaccine, some parents report anecdotal data suggesting such a link. Research indicates that some children with autism have immune abnormalities on laboratory testing. Operating on the premise that their immunodysregulation may be associated with the pathogenesis of autism, I tested for the possible beneficial effects of intravenous gamma globulin therapy on these children.

Ten years ago, my practice and research focused almost exclusively on preventing mother-to-child HIV transmission and caring for children infected with the virus. It was because of this much-publicized work that I was contacted by Ray Gallup, president of the Autism Autoimmunity Project, an organization raising funds to support research into the prevalence and source of immune dysfunction in children with autism, to investigate a possible link between the disorder and specific childhood vaccines. Mr. Gallup was also looking for a physician to provide immunological treatment for his son, Eric, whose autism symptoms seemed to emerge after immunization with the MMR vaccine. At that time, I knew little about the disorder, and had never considered a link between autism and immune dysfunction. Mr. Gallup had done extensive reading on the subject, and wanted my professional opinion on whether Transfer Factor, a product derived from lymphocytes, or intravenous gamma globulin (IVIG), used to boost the damaged immune systems of HIV-infected children, might benefit children with autism whose immune systems were malfunctioning.

I reviewed the research on autism. While I was not convinced of a direct link between immunizations, abnormal immune responses and autism, there was evidence suggesting that some children with autism also have immune system problems. I agreed to evaluate Eric for immunodeficiency and give recommendations concerning immune based therapy. Much to my surprise, the child did have abnormalities in his humoral immune system, and I recommended IVIG to reduce the number of infections he was contracting which required antibiotics. I did not know if this would impact any of his autism symptoms. Monthly gamma globulin treatments administered over a period of six months did reduce the number of Eric's infections and, consequently, his need for antibiotics. The family said that some of Eric's autism symptoms also improved.

Based on my initial experience with Eric, I evaluated more than 80 children with autism and found that 27 had immune abnormalities on laboratory testing. Operating on the premise that their immunodysregulation may be associated with the pathogenesis of autism, I tested for the possible beneficial effects of IVIG replacement therapy in children with autism who also demonstrated humoral immunodeficiency. This open-label, IRB-approved clinical trial enrolled 27 autistic children ages 2 to 10 (median age 3 years). They were treated with IVIG (0.4 to 1 gm/kg/dose) every three weeks for 6 to 18 months secondary to low serum IgG (5/27), IgG subclass deficiency (12/27), or functional antibody deficiency with recurrent infection unresponsive to conventional therapy (10/27). In 4 of 27 patients, serum IgM levels were higher than normal prior to IVIG therapy. Frequency of CD4+ T cells, CD19+ B cells and NK (CD16+/CD56+) cells were lower than normal range in 7/27, 4/27 and 5/27 patients, respectively.

Changes in clinical features of autism observed by physicians and parents at the conclusion of therapy were noted. Some common recurrent infections were better controlled: otitis media (19/27); upper respiratory infections (11/27); and sinopulmonary infections (9/27). Antibiotic therapy was reduced in 20/27 children during the IVIG treatment. Parents and physicians also reported an improvement in autistic behaviors in 21/27 children, and one child had reported improvement in autistic features without improvement in recurrent infections.

While the information generated from the study was not conclusive, it suggested that children with autism who have evidence of humoral immune dysfunction may benefit from IVIG therapy, which can reduce the number of their recurrent infections, which may, in turn, improve clinical features of autism.

More controversial was the finding that several of the children with autism who had immunodeficiency also had higher titers to measles antibodies after immunization with the MMR vaccine compared to children without autism following vaccination. My laboratory is still evaluating some

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of the data to determine if these children with autism did indeed have abnormal responses to MMR.

Most epidemiological studies fail to confirm any general link between autism and the MMR vaccine. However, we are still investigating the possibility that there is a subset of children with autism whose immunologic response to measles immunization is linked to their disorder.

James M. Oleske is one of the country's foremost pediatric HIV/AIDS specialists. He is the Francois-Xavier Bagnoud Professor of Pediatrics and director of the Division of Pulmonary, Allergy, Immunology and Infectious Diseases at UMDNJ-New Jersey Medical School (NJMS), and medical director of the FXB Center at UMDNJ, one of the nation's largest treatment centers for childhood HIV/AIDS. He earned his MD from NJMS in 1971 and an MPH from Columbia University. After completing specialty training in pediatric allergy, immunology and infectious diseases at Emory University in Atlanta, he returned to join the faculty of NJMS. Despite his diversified clinical and research interests, he has spent the last 15 years working in the area of HIV infection and AIDS. Dr. Oleske has been a strong advocate for children, adolescents and women with HIV infection, and has testified before Congress on diverse AIDS-related issues. He has served on many advisory boards, including the NIAID Review Committee and the CDC Committee on Pediatric AIDS; and has written more than 100 peer reviewed articles.

INFORMATION FROM THE STUDY, WHILE NOT CONCLUSIVE, SUGGESTED THAT CHILDREN WITH AUTISM WHO HAVE EVIDENCE OF HUMORAL DYSFUNCTION MAY BENEFIT FROM IVIG THERAPY.

Over the years, I've learned several things:

- There seems to be a real and as yet unexplained increase in children being diagnosed with autism.
- Families of these children are remarkable advocates, despite marked decreases in their quality of life.
- Autism is a complex disorder linked to genetics, but it appears to be triggered by heterogeneous environmental factors. One of these triggering factors may be due to immune dysfunction and an abnormal inflammatory response.

Promising research is also being conducted by Dr. Harumi Jyonouchi from our department at NJMS into the link between autism, abnormal inflammatory responses and food allergies. Her article can be read on page 11.

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Autism Research Moves Forward

BY STUART D. COOK

Autism spectrum disorder is being diagnosed with dramatically increased frequency in New Jersey and nationwide, but there is considerable debate about whether this is a result of rising incidence or increased awareness.

We are all certainly more aware of autism than we were just 10 years ago. Now that its diagnostic criteria have been codified, and medical detection and recognition have improved, more cases of the disorder are being uncovered. However, I believe that we are also seeing more cases of autism because of a real increase in the number of individuals affected, suggesting that important environmental factors may serve as triggers in those with genetic susceptibility.

Autism is a complex disorder and it will take the dedicated efforts of researchers in many fields to uncover its root causes. Some of UMDNJ's most talented investigators are on the case. Toxicologists, neuroscientists, geneticists, developmental pediatricians, environmentalists, psychiatrists and immunologists are collaborating to understand how to prevent the disorder, and also how to improve the quality of life of those with autism.

Fortunately, public and private funds to underwrite this research have become available, not the least of which is funding from the State of New Jersey. The combination of new technology, talent and substantial financial support offers hope that real progress will be made in the near future.

The University is playing a pivotal role in New Jersey's autism efforts because it can work statewide and across disciplines, as well as collaboratively with researchers at other universities, institutes and in industry. Collaboration is one of the key components in moving complex research forward more quickly and comprehensively. UMDNJ's many productive partnerships with the pharmaceutical industry, particularly in diseases of the brain and nervous system, including multiple sclerosis, stroke, schizophrenia, Alzheimer's disease and autism, are yielding new and better therapies for those affected.

This issue of *UMDNJ Research* introduces some of the University's outstanding researchers who together are moving our understanding of autism and the care we can offer individuals and families to the next level. I feel confident that with so much serious attention and work, some of the answers we seek will soon be forthcoming.

Stuart D. Cook

Stuart D. Cook, MD
President
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