Deconstructing a Complex Disorder

A GW researcher applies order and simplification as a new step toward understanding autism

On a Wednesday last April, Valerie Hu, Ph.D., professor of Biochemistry and Molecular Biology at GW’s School of Medicine and Health Sciences (SMHS), eagerly anticipated the release of an autism spectrum disorder (ASD) study that she authored and that appeared in the online science journal *PLoS One*. The study added to an already large body of literature linking specific genes to autism, but Hu emphasized that her genome-wide analysis provided “a new twist.”

Hu’s approach addressed the immense variability of autism, a disorder that affects one out of 110 American children, according to the Centers for Disease Control and Prevention. Autism’s severity and symptoms vary drastically: They include language deficiency, repetitive behaviors, and restricted social interest; some individuals also exhibit epilepsy, sleep disturbance, weak muscle tone, and immune system dysfunction. “When I talk to parents about studying autism,” Hu says, “I tell them it’s like digging your hand into a bowl of mixed fruit and picking out one specific thing.”

Experts agree that autism is a multi-gene disorder, and each gene inflicts a small effect that is compounded by environmental factors. The unique jumble of symptoms emerges in almost a case-by-case basis. “This heterogeneity in symptoms, severity, etc., is the major problem,” Hu explains. “Most scientists and geneticists recognize this, but little has been done to tease the heterogeneity apart. The result is increased ‘noise’ in the biological or genetic data.”

**THE BREAKDOWN**

Hu’s recent study seeks to squelch that noise and gain a clearer signal by systematically grouping individuals with autism into subgroups according to similarity of severity scores from the Autism Diagnostic Interview–Revised (ADI-R), a behavioral diagnostic assessment. These scores,
along with the corresponding individual’s genetic data, were obtained from a large study led by faculty at the Children’s Hospital of Philadelphia.

Hu clustered nearly 3,000 individuals into four distinguishable ASD groups that ranged in severity from mild to severely language impaired, with the hope of determining whether the behavioral subtypes could be associated with different genetic variations.

This study identified 18 novel genetic variants or single nucleotide polymorphisms (SNPs) that were associated with one or more subgroups of autism. Interestingly, the SNPs were in regions of DNA that didn’t code for proteins, suggesting that they may instead regulate gene expression. The value of dividing individuals into subgroups for the genetic analyses was underscored by the fact that no significant SNPs were identified when the cases were combined into a single group (Hu’s “bowl of mixed fruit”). “To associate genotype with behavioral phenotype is what you want for diagnostics,” Hu says. “You want to pick out not just risk for autism, but also risk for a type of autism.”

Anthony-Samuel LaMania, Ph.D., professor of Pharmacology and Physiology and director of GW’s new Institute for Neuroscience, stresses the importance of taking into account autism’s variability when connecting observable deficits to their underlying genome. “It’s not as simple as making a clinical diagnosis of autism and then finding a gene,” he says. “There’s a necessity in identifying each behavioral realm and associating it with a number of genomic changes. These sorts of groupings will help with that.”

PATTERNS EMERGE
The success of this genome-wide genetic analysis in identifying subtype-dependent SNPs supports this subtyping strategy, which Hu and her colleagues had previously applied to genome-wide gene expression analyses. In a 2009 study that analyzed more than 40,000 RNA transcripts per sample, both overlapping and unique genes were revealed among three of the subtypes examined.

Among the genes shared between the severe and mild subtypes were those linked to cell death, inflammation, embryonic development, memory, learning, and muscle tone. Unique to the more severe ASD group were genes associated with responses to painful and normal stimuli, muscle rigidity, epilepsy, and circadian rhythm.

Altogether, Hu views her findings as necessary steps toward targeted therapies in autism. “In order to develop a personalized medicine approach to treating a complex disorder,” she says, “you need to define what the defect is.”

IT’S PERSONAL
With good reason, Hu decided in 2004 to pivot her research acumen toward autism. “I have a son who’s affected. That’s my driving motivation,” she explains. As a parent and a scientist, she is all too aware of how the diagnosis of autism relies solely on behavioral assessments, since reliable biomarkers have yet to become a reality. “Even though autism is said to be highly heritable, there’s still no genetic or molecular marker,” she says. “So one of the goals of our study was biomarker identification.”

With regard to the diagnostic landscape of autism, Rakesh Kumar, Ph.D., professor and the Catharine Birch & William McCormick Endowed Chair of the Department of Biochemistry and Molecular Biology at SMHS, couldn’t agree more about the need for a quantitative tool. “There is no yardstick to measure autism against,” he says. “There is a lot of subjectivity that comes with complex disorders, and they cannot be assigned to a single gene product. This is where Dr. Hu’s work will be able to fill in the niche.”

Absent a biological understanding of autism, treatment options are limited and vulnerable to conjecture. “Right now they’re just throwing all these different psychotropic drugs at autistic kids,” Hu observes. “My son has been prescribed more than 20 different drugs. None of the drugs have worked, and many have had intolerable side effects, so now he’s not on anything.”

Ideally, making a diagnosis based on peripheral biomarkers stems from an understanding of how the detected changes relate to the nervous system, LaMania points out. “The diagnostic strategy requires asking whether peripheral change in a non-neuronal cell type represents something significant in the nervous system,” he adds.

A FRAMEWORK FOR THE BIG PICTURE
Making sense of autism means unraveling its many causes and seeing how they relate. Hu likes to construe it as a multi level pyramid with the base representing the observable symptoms, or phenotype. The next level is the gene expression profile, above which is the genetic hardware that you’re born with as well as the ways in which that hardware is interpreted, or epigenetics. At the top are the environmental and biological triggers, such as hormones and toxins. “Even though autism is highly genetic,” Hu notes, “the question is what environmental factors impact the genes. That’s the big picture.”

Because genetically identical twins who differ in their diagnosis for autism show different gene expression profiles, Hu thought it was a good idea to look at DNA methylation, an epigenetic process whereby methyl groups attach to DNA to dampen gene expression.

In a 2010 study, Hu and Anh Thu Nguyen, then a graduate student working in Hu’s lab, identified the retinoic acid-related orphan receptor alpha gene, or RORA, as a gene that’s altered by methylation in samples from autistic individuals. RORA mutation has been linked to
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an abnormal cerebellum (the region of the brain that's responsible for coordination and muscle tone) and deficiency in a type of neuronal cell (Purkinje), which has been the earliest and most consistent brain abnormality observed in autism.

This February, Hu and her collaborators — Tewarit Sarachana, a GW Institute for Biomedical Sciences Ph.D. candidate, Minyi Xu, a master’s degree student in Biochemistry who now serves as a research assistant under Hu; and Ray-Chang Wu, Ph.D., assistant professor of Biochemistry and Molecular Biology — reported that RORA is regulated in opposite directions by male and female sex hormones (male hormones repress it), and also that RORA regulates aromatase, an enzyme that converts testosterone to estrogen. The study further showed that both RORA and aromatase levels are reduced in the brain tissues of autistic individuals, thus linking observations initially made with non-neuronal tissues to changes in the brain. These findings are significant because they provide an explanation for the higher amount of testosterone often seen in children with autism, which some researchers blame for the 4:1 sex difference between boys and girls with the disorder.

"I think females are more protected because estrogen cannot only elevate RORA expression, but also compensate for a deficiency in RORA by activating the estrogen receptor which controls some of the same genes that are regulated by RORA," Hu says. "That's what I think is the beauty of the work with sex hormones. It really helps explain the higher levels of testosterone, as well as the sex bias in autism."

EYES ON THE PRIZE, FEET ON THE GROUND
Hu explains her work like a driven detective searching for clues. Kumar describes her as a principal investigator who single-handedly put GW on the map for autism research. "Her work," he says, "will collectively help to further refine the autistic diagnostic kit based on objective, quantitative tests."

Hu hopes her work will one day spur better diagnosis and treatment of autism. "Sometimes when you think too far ahead, it's so complex," she reflects. "You wonder whether you'll get to a point where you can really help."

LaMantia agrees that solving the riddle of autism will be a piecemeal, formidable challenge. "Autism research is moving forward right now," LaMantia observes, "but it is going to be complicated, and it will move forward ever so slowly."

It's understandable that parents of autistic children would find such an outlook sobering. After Hu spoke about autism at a recent conference in Utah, a parent asked if giving estrogen to her affected son would alleviate symptoms. As a parent and a scientific expert, Hu could relate to the eagerness. But she framed her answer pragmatically: "You have to let research sort that out."

Taped to Hu's computer monitor is a fortune cookie message: "Your present plans are going to succeed." It’s a sage reminder for any investigator who has a hunch he or she is on the right track. ■