PROBING HUNTINGTON'S ORIGINS:
Computational Approaches May Lead to Earlier Interventions

By Esther Landhuis

Uncontrolled writhing and jerking. Poor judgment. Depression and irritability. It's hard to imagine how this unnerving mix of movement, cognitive and psychiatric problems arises from a single genetic blip—one that plops unusually long stretches of the amino acid glutamine in the culprit protein for Huntington's disease (HD). Researchers who study this brain disorder are still puzzling over how the rogue molecule causes so much to go awry.

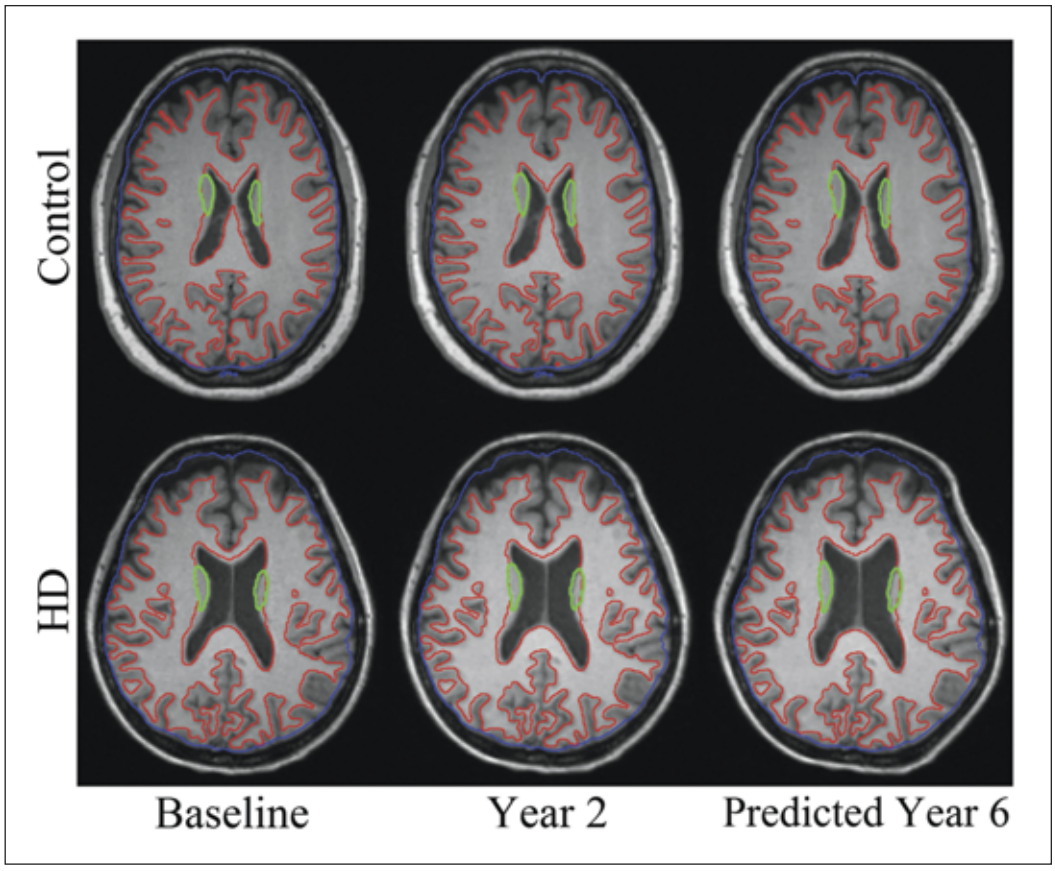
What they do know is that people who inherit the huntingtin gene mutation are sure to develop the disease and die of it. In Western nations, the disease strikes one in every 10,000 to 20,000 people, destroying neurons in areas at the base of the brain known as the basal ganglia. On magnetic resonance imaging (MRI) scans that measure brain volume, regions of the basal ganglia appear heavily shrunken in Huntington's patients, relative to normal adults. By the time symptoms appear, "they've already lost a tremendous amount of brain structure. It's hard to regain that," says **Hans Johnson, PhD**, assistant professor of psychiatry and biomedical engineering at the University of Iowa Carver College of Medicine in Iowa City.

While structural MRI measures of brain volume can be useful for understanding the biological degradation that has occurred at the time of diagnosis, Johnson and other researchers are now using state-of-the-art neuroimaging and computational approaches to look much earlier in the disease process. Churning out four- or five-dimensional data, these newer methods burn through 1,000 to 2,000 times as much mathematical and computational power as volumetric MRI. But they are yielding valuable clues—subtle changes in circuitry and function that seem to lurk within the brain for years, perhaps even decades, before symptoms become serious enough to prompt a doctor's visit. The researchers hope that teasing out these early abnormalities will guide them toward new strategies to protect the brain from impending damage. Other scientists are taking a different tack, using bioinformatics to uncover other genes and pathways that may be linked to Huntington's pathogenesis.

Neuroimaging: Structural and Functional Brain Changes

One of the field's biggest surprises—which also may explain why Huntington's symptoms remain under the clinician's radar for so many years—is the brain's incredible ability to adapt. "We can function pretty darn well by recruiting non-traditional parts of our brain to work on tasks," Johnson says. For instance, early trouble in memory circuits can trigger the brain to rewire itself in ways that bypass problem spots by activating neurons from other areas. Some researchers believe this process kicks in as a compensatory process to help people on the verge of Huntington's retain function in the face of early degeneration.

Such insights come from studies that use functional MRI (fMRI)—a technique that measures brain activity by detecting changes in blood flow. A typical functional neuroimaging session produces 20 gigabytes of raw data, Johnson notes. "In addition to drawing a ruler on the screen and counting the number of voxels in a region, we need to correlate the task being run with other



The brains of HD patients (bottom) show progressive expansion of the ventricle (large dark area in the middle) and thinning of the caudate (green outline) from baseline (left) until two years later (middle). And researchers have extrapolated that change six years into the future as well (right). In contrast, a control subject does not show much change at all (top). Animations of the series shown here are particularly compelling and available online at http://www.cs.utah.edu/~jfishbau/docs/ctrl_and_hd.gif. Courtesy of James Fishbaugh.

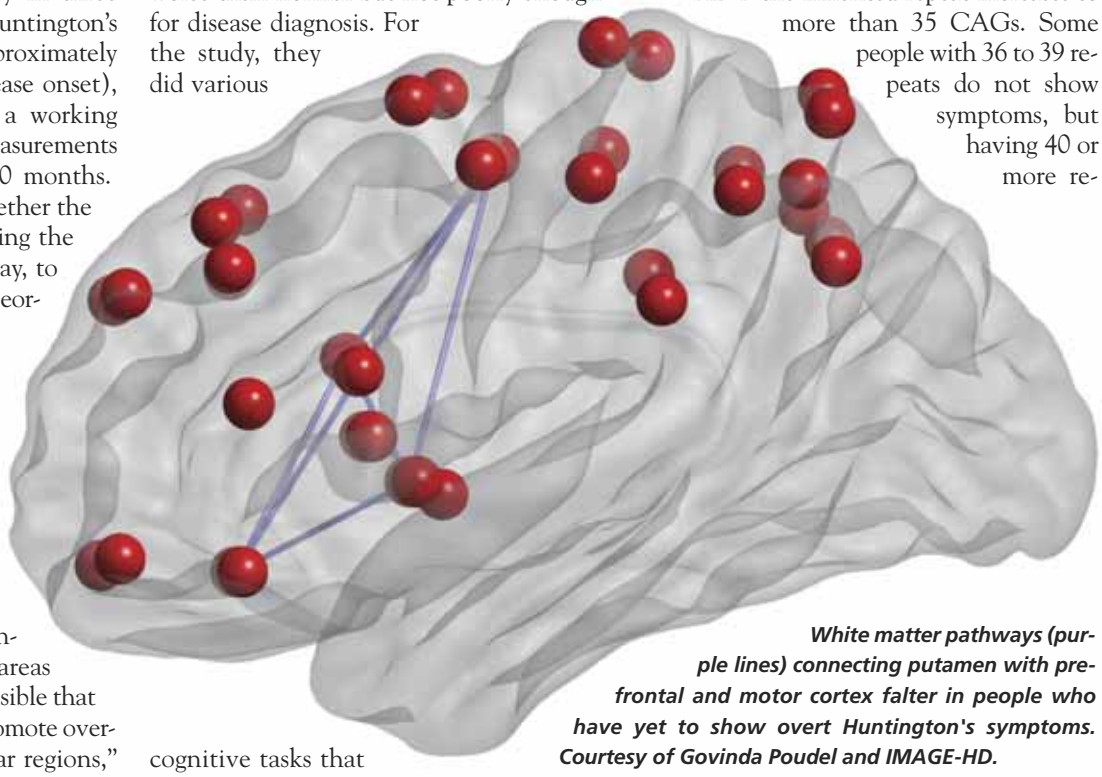
variables such as heartbeat and breathing rate, in order to get to the signal that we then have to extract mathematically with the task being performed."

Prior fMRI analyses support the idea that functional reorganization occurs early in disease, but results have been mixed—some showed a boost in brain activity while others portrayed a decrease. Moreover, past studies have been cross-sectional, analyzing data on patient subgroups at just one point in time. Researchers led by **Nellie Georgiou-Karistianis, PhD**, a cognitive neuroscience professor at Monash University in Melbourne, Australia, investigated the compensation issue more rigorously in a longitudinal study known as IMAGE-HD. The team used fMRI to monitor brain activity in three groups of people—those with Huntington's symptoms, mutation carriers (approximately 15 years prior to estimated disease onset), and healthy controls—during a working memory task. They made the measurements at baseline, 18 months, and 30 months. "Our focus was to determine whether the brain is able to compensate during the 'pre-manifest' stage—in some way, to help individuals stay on task," Georgiou-Karistianis says.

As her team reported in a November 16, 2013, *Brain Structure & Function* paper, the answer seems to be yes. Over the 30-month study, the fMRI scans showed subcortical and cortical areas firing more intensively in people on the verge of symptom onset, compared with the other groups. In addition, connections between those brain areas appeared to be faltering. "It is possible that the reduced connectivity may promote overcompensation in these particular regions," Georgiou-Karistianis says, noting that these areas could be used as targets for future pharmacological intervention. "The overcompensation might also be a response to the structural brain changes that are also happening very early during the disease."

Some of those abnormalities may show up with diffusion-weighted imaging (DWI), a newer type of MRI that requires massive computing power to visualize tissue architecture. Instead of a single picture of a brain structure, DWI generates some 80 high-resolution sub-pictures that help scientists model the vibration of water molecules. Those vibrations give insight into the integrity of the underlying tissue. Using the analogy of an electric circuit, fMRI measures the strength of signals whereas DWI gives insight into the integrity of the wires that carry the signals. For example, is the insulating shell around the wires intact, or does it have holes where it's rubbed against something else? Does the insulation work properly? The "insulation" that surrounds the axonal projections of neurons is known as white matter. Diffusion MRI helps researchers determine how the white matter bundles are organized, whether they're packed densely or loosely, for example.

A team of researchers led by **Jane Paulsen, PhD**, a professor of psychiatry at the University of Iowa, Iowa City, used diffusion-weighted imaging to measure white matter changes in the prefrontal cortex of people with prodromal Huntington's disease. These individuals perform slightly worse than normal but not poorly enough for disease diagnosis. For the study, they did various cognitive tasks that required them to identify words or colors, or link numbers together. In a paper published in April 2013 in *Human Brain Mapping*, Paulsen and colleagues reported that myelin sheath integrity and other white matter measures seemed to track with cognitive readouts in those areas. "For example, in the word-finding task, white matter deficits showed up in the part of the brain needed for word processing," noted Johnson, who was a coauthor on the research.



White matter pathways (purple lines) connecting putamen with prefrontal and motor cortex falter in people who have yet to show overt Huntington's symptoms. Courtesy of Govinda Poudel and IMAGE-HD.

Bioinformatics: Other Genes and Pathways

While neuroimaging afficionados strive to understand the earliest stages of Huntington's by probing the brain's inner workings, others are coming at the issue using bioinformatics. Their computational strategies are scouring massive gene-expression datasets for pathways that are altered by mutant huntingtin.

The huntingtin protein is expressed in many cell types, but scientists don't understand quite what it does. It interacts with a huge number of proteins and has structural features found in organizers of molecular complexes.

The huntingtin (*HTT*) mutation is also intriguing. All of us have CAG (cytosine-adenine-guanine) trinucleotide repeats in our huntingtin gene. In fact, we can have up to 35 *HTT* CAG repeats and still be considered normal. In rare cases, people with 27 to 35 *HTT* CAG repeats can have children with HD if the inherited repeat increases to more than 35 CAGs. Some people with 36 to 39 repeats do not show symptoms, but having 40 or more repeats virtually guarantees disease. The size of the *HTT* CAG repeat mutation correlates inversely with age of symptom onset—in general, the more *HTT* CAG repeats, the earlier a person will develop disease.

The prevailing view holds that the *huntingtin* mutation acts through full-length expression of the gene. "We're not dealing with a protein that loses function. It's either gaining function or getting dysregulated," says **James Gusella, PhD**, who directs the Center for Human Genetic Research at Massachusetts General Hospital in Boston.

Until recently, researchers doing gene expression studies to understand the effect of the *HTT* CAG repeat assumed they would get the strongest signal by analyzing extreme populations—that is, genomes with

strong disease mutations and those devoid of mutations. Hence, they have compared two groups: patients with HD symptoms and normal patients (controls). These dichotomous analyses failed to take into account differences in CAG repeat length.

Gusella and his colleagues therefore took a different analytical approach: They correlated gene expression across the continuum of *HTT* CAG repeats from low to high (15 to 92) in 97 lymphoblastoid cell lines (the training set). They used the results to mathematically predict CAG repeat numbers in a set of 10 cell lines (the test set). The proof-of-concept study showed that differences in transcript levels can detect the continuous effects of increasing *HTT* CAG repeat length and provide an approach to discovering factors contributing to the pathogenic process, which also increases with *HTT* CAG repeat length. The expression changes appeared in genes involved in chromatin remodeling, energy metabolism and axonal transport, suggesting that CAG repeats have downstream consequences on molecules involved in these pathways. However, how these systems connect is not clear, Gusella says. "The big picture hasn't yet come together." The work appeared in *Human Molecular Genetics* in April 2013.

Future: Bigger Datasets

In the long run, gaining a more complete picture of Huntington's disease progression will require a pooling of many different types of studies—gene expression, fMRI and DWI and others, Johnson says. "Investigating it jointly rather than independently is really where the future promise is." □

DESIGNING LIFE'S LAYERED CIRCUITS: Tools of the Trade

By Sarah C.P. Williams

In synthetic biology labs around the world, brainstorming has often begun at the same place: in front of a whiteboard. Marker in hand, researchers jot down the parts needed to form a new circuit, draw lines and arrows to show how they interact, and scrawl notes about how to assemble the parts into an appropriate whole.

"It's usually based on intuition, and what we know has worked in the past," says **Timothy Lu, MD, PhD**, who heads up the Synthetic Biology Group at the Massachusetts Institute of Technology.

The whiteboard has been used to design many novel genetic programs—whether aimed at turning bacteria into biosensors or forming networks of enzymes to churn out a particular product. But the way of the whiteboard might be fading. As circuits become more and more complex, and researchers move toward the design of larger networks and whole-cell programs, it's becoming harder to manage all the required parts for a new project in hand-written dry erase.

"When I was looking at a simple circuit with two inputs, I could by hand iterate through all the possible states of the system," Lu says. "Now, I'm interested in things with six or eight inputs, and intuition starts to fail."

Costas D. Maranas, PhD, professor of chemical engineering at Penn State University, concurs. While synthetic circuits of a decade ago had a single switch and just a few inputs to alter genes, Maranas is trying to reconstruct and regulate the entire repertoire of pathways involved in a microbe's metabolism.

And it's not just the number of switches that adds complexity. Adding new enzyme activity into a bacterium is more complicated than just adding the enzyme. Take nitrogenase, for example, which Maranas and collaborators at Washington University would like to be able to control within a cyanobacterium. Getting the right levels of nitrogenase activity, he adds, doesn't just mean having the right levels of gene and protein expression, but also accurately reproducing the light to dark transitions and providing sufficient energy in the form of ATP to power the nitrogenase.

To help manage this complexity, researchers are developing, refining, and applying computerized design programs that track the parts involved in their systems and pinpoint the best method to assemble a new circuit. There's not yet one program that fulfils the dream of "plug and play" biology—where a few simple clicks choose the parts for the essential biological circuits and, voilà, synthetic life! But several programs are emerging as crucial to the field.

Inspired by Engineering

In the mid-2000s, **Jean Peccoud, PhD**, a computational synthetic biology researcher at Virginia Tech's Bioinformatics Institute, was working on recreating the genetic networks that control cell division in yeast. Like other synthetic biologists, Peccoud viewed the components of the network—genes, promoters, ribosome binding sites, and terminators, to name a few—as discrete parts, with defined functions, that could be shuffled around between networks. But he realized that no software existed that could track which parts worked together, guide how the parts could be plugged into genetic circuits, and model how a proposed circuit would function.

"It seemed reasonable to assume that synthetic biology would need some computer-aided-design tools just like any other engineering discipline," says Peccoud. CAD programs are heavily relied on by electrical and mechanical engineers, for example, to design electrical circuits or structures on the computer before they're created and tested.

So his lab began developing such a program for biology. The result: GenoCAD, an open-source, synthetic biology CAD software. GenoCAD manages lists of genetic parts and gives users an interface where they can set design rules, apply them to their system and then assemble genetic parts into plasmids. It also includes a simulation engine to test new circuits.

In the December 2013 issue of *ACS Synthetic Biology*, Peccoud and two collaborators describe using GenoCAD to create a set of