



GLADSTONE RESEARCHERS RESOLVE KEY HUNTINGTON'S DISEASE MYSTERY IN *NATURE* COVER STORY

Use Innovative Robotic Microscopy to Image Molecular Processes in Neurons

A mystery long associated with Huntington's disease has been resolved by a team of researchers at the UCSF-affiliated Gladstone Institute of Neurological Disease, thanks to a specially designed microscope that allows researchers to track changes in cells, including those associated with neurodegeneration, over long lengths of time.

As reported in the cover story in the current issue of *Nature* (Oct. 14, 2004), the team determined that abnormal deposits of mutant huntingtin protein, which appear in the brains of all Huntington's disease patients, aren't the cause of neuronal death. Scientists know that mutant huntingtin protein is responsible for the disease, but they have not known in what form it wreaks its havoc. They haven't known, for instance, whether the abnormal deposits of the protein, known as "inclusion bodies," were, themselves, causative, protective or incidental to the disease. In the current study, the Gladstone team determined that inclusion bodies are a beneficial coping response, possibly sequestering mutant huntingtin protein, thereby reducing levels of the protein elsewhere in the neuron, and thus prolonging neurons' survival.

The finding suggests that mutant huntingtin protein inflicts its damage in some form other than as inclusion bodies, which are insoluble, or resistant to being dissolved in liquid. Investigators may now focus attention on the possibility that the real culprit is a more soluble form of mutant huntingtin spread throughout the neuron, or nerve cell, among other theories.

"We are very excited by these results," says lead investigator Steven Finkbeiner, MD, PhD, an assistant investigator at the Gladstone Institute of Neurological Disease and assistant professor of neurology and physiology at University of California, San Francisco (UCSF). "They will help us to better focus efforts to identify the mechanisms by which the huntingtin protein causes Huntington's and may add to the understanding of other neurodegenerative disorders."

Traditionally, scientists have tried to illuminate the role of the mutant protein within neurons by taking one-time snapshots of individual cells, a slow process that doesn't allow researchers to track changes in any given cell over time. Beyond slowness, a fundamental problem with this conventional approach is that the snapshots are not only taken at different times but also each image is of a completely different population of cells than the other. Scientists have tried to use these images to piece together theories of disease progression, but have had great difficulty interpreting their results because of the lack of continuity between images.

To address these issues, Finkbeiner developed an automated microscope that allows researchers to track changes in individual neurons over time, thus enabling them to identify factors that predict the fate of the cell.

"With this new technology, we can examine neurons well before they die, make measurements of whatever we wish, and then determine which factors have prognostic value, whether they predict survival or neurodegeneration, and how strong the prediction is. This is a powerful new way to guide our investigation into the underlying mechanisms of neurodegeneration," he explains.

In their study, the scientists introduced fluorescently tagged versions of huntingtin protein into neurons. They then used the robotic microscope to monitor the accumulation of the abnormal protein into inclusion bodies, as well as to monitor the levels of intracellular huntingtin protein, and the length of survival of thousands of individual cells over time. Sophisticated statistical techniques for survival analysis were then used

to determine whether a particular abnormality predicted early death and might be pathogenic, or predicted longer survival and might be beneficial.

The findings suggest, says Finkbeiner, that inclusion bodies lock up mutant huntingtin in other parts of a cell and keep it from interfering with the rest of the neuron in ways that can trigger cell death. These findings provide evidence that inclusion bodies in Huntington's disease, and possibly other neurodegenerative diseases, help neurons cope with toxic proteins and prevent neurodegeneration.

The approach developed by the Finkbeiner group—combining the use of a robotic microscope with powerful techniques of statistical analysis—could also be used in studies of other neurodegenerative diseases characterized by the accumulation of cellular proteins, including Alzheimer's disease, prion diseases, amyotrophic lateral sclerosis (Lou Gehrig's disease), Parkinson's disease, and a group of nine so-called polyglutamine diseases of which Huntington's is the most widely known.

Moreover, the approach could be used to measure the nature and magnitude of the relationship between any two biological events within a cell that are separated by time. With this tool, researchers can begin to answer such fundamental questions as:

- Is there a relationship at all, or are the two events simply coincidental?
- If there is a relationship, is one event possibly the cause or the effect of the other?
- If it is a cause, is it a minor determinant or a major one?

These are questions that recur in all aspects of cell biology.

As Professor Harry T. Orr of the University of Minnesota explains in a companion *Nature* commentary, "In the long term, strength of this study lies in the approach itself. The capability to determine if a cellular feature of a disease is pathogenic, beneficial, or merely incidental to a disease process will be of considerable advantage for understanding disease mechanisms. Will the results reported here end the debate on the pathogenic role of inclusion bodies in the polyglutamine diseases? If not, one wonders what would."

Huntington's disease is a hereditary, progressive neurodegenerative disorder characterized by the development of emotional, behavioral, and psychiatric abnormalities, loss of intellectual and cognitive functioning, and motor disturbances. Although symptoms typically become evident during the fourth or fifth decades of life, the age at onset is variable and ranges from early childhood to the 70s or 80s. It's named for the American physician who initially described the condition in 1872.

The paper, "Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death," was co-authored by Finkbeiner and fellow GIND staff members Montserrat Arrasate and Siddhartha Mitra; Erik S. Schweitzer of the Brain Research Institute, UCLA; and Mark R. Segal of the Division of Biostatistics, UCSF. Primary support for this work was provided by the National Institute of Neurological Disorders and Stroke (NINDS), part of the National Institutes of Health (NIH). Additional support was provided by the National Institute of Aging within the NIH, and the J. David Gladstone Institutes.

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