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Researchers Reveal Molecular Origin of a Spectrum of Human Axon Guidance Disorders

Scientists who searched across five continents for patients who have a rare eye movement disorder have finally identified its genetic root. In the process, they have found that specific mutations in this new disease gene can cause additional errors in neurodevelopment and neuronal survival, leading to the definition of a new series of neurological disorders that interfere with nerve cells' ability to wire up properly. They now refer to these as the TUBB3 syndromes.

The study, which is published in the January 8, 2010, issue of the journal *Cell*, reveals these syndromes can be caused by any one of eight mutations in the TUBB3 gene, which encodes a structural protein in neurons. The collaborative effort was led by Howard Hughes Medical Institute (HHMI) investigator Elizabeth Engle of Children's Hospital Boston.

Engle, a physician trained in pediatrics, neurology, and neuropathology, has previously published pivotal studies identifying genes underlying other eye movement disorders. She found that these resulted from errors in the development of motor neurons or their long processes, called axons, that connect the eye muscles to the brain.

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- Elizabeth C. Engle

In the new study, Engle's team focused on children born with congenital fibrosis of the extraocular muscles type 3 (CFEOM3), who have drooping eyelids and are unable to move their eyes fully. CFEOM3 can be inherited as

a dominant disorder. To begin the search for the cause of CFEOM3, Engle says, it was essential to find and characterize the families of patients with the rare disorder, and that required a major international effort.

“This paper includes 29 families who harbor *TUBB3* mutations,” says Engle, whose collaborative team compiled detailed family histories of study participants from the USA, Europe, Turkey, Venezuela, and Australia. “We had a rare opportunity to collaborate with 36 clinicians around the world for this particular study, which was a key to its success. The clinical collaborators identified the families, enrolled participants in the study, and provided us with essential clinical data that permitted us to later correlate specific mutations with specific clinical findings.”

The clinical data revealed that while some children with CFEOM3 are otherwise normal, others had social, behavioral or intellectual impairments, and some went on to develop nerve degeneration that resulted in weakness and numbness in their arms or legs. In addition, MRI analysis showed that nerve bundles beyond those that help control eye movement had not formed correctly, particularly nerve bundles that connect the left and right sides of the brain. These MRI findings correlated with developmental disabilities in affected individuals.

Engle’s lab had previously discovered that a region spanning about 50 genes at the tip of human chromosome 16 contained an unknown gene that caused CFEOM3. In the current study, her group evaluated DNA from these 29 affected families to pinpoint eight different mutations in the *TUBB3* gene as the culprit.

“What we found to be particularly remarkable was that multiple unrelated families could share the same mutation in the *TUBB3* gene, and the affected members in those families had similar clinical presentations,” says Engle. “While some mutations caused isolated CFEOM3, others caused CFEOM3 with a peripheral axonal neuropathy, or CFEOM3 with developmental disorders. Thus, given a particular individual’s clinical presentation, we could often accurately predict the mutation he or she harbored. Moreover, the clinical and MRI findings in these participants suggested that the primary error was in the guidance of axons, the long projections of neuronal cell bodies that connect one neuron to another or to a muscle.”

TUBB3 encodes a protein called β tubulin isotype III. Tubulins make up the intracellular scaffold known as the microtubule network. The specific β tubulin that is impaired in these human syndromes is found exclusively in nerve cells, where microtubules form tracks on which proteins are moved from the cell body out through long projections called axons. Growing axons must navigate past neighboring neurons as they form connections with other cells. This requires that the microtubule track inside the axon, particularly at its leading tip, be continuously laid down, ripped up, and laid down again.

With the gene identified as *TUBB3*, Engle's group forged ahead with experiments in mice and yeast to try to determine why specific mutations in this particular β tubulin gene produced CFEOM3 and associated neurological symptoms. Engle says first author Max Tischfield, a graduate student in her lab, was a driving force behind the *TUBB3* experiments that built on the study's initial genetic steps.

First, in a mouse model mimicking the most common human CFEOM3 mutation, they observed that neuronal cell bodies were in the right place anatomically in the brains of embryonic mice but that those cells did a poor job of projecting axons to eye muscles or to other regions of the brain.

"Similar to the human MRI data, these mouse experiments supported a defect not of neuron migration," says Engle, "but of axon guidance" -- the biochemical activities that allow nerve cells to wire up to the right target cells.

"The microtubule network must be dynamic to allow an axon to navigate through a three-dimensional space," explains Tischfield. "We found that the primary effect of *TUBB3* mutations was to alter those dynamic properties and compromise the ability of microtubules to grow or shrink."

The clincher came when Tischfield mutated the gene encoding yeast β tubulin in ways mimicking the eight *TUBB3* mutations the researchers had found in humans, studies greatly aided by collaboration with David Pellman, an HHMI investigator at Dana-Farber Cancer Institute, and Pellman's former postdoctoral fellow Mohan Gupta, now an assistant professor at the University of Chicago. The experiments demonstrated that all of the human mutations resulted in more stable yeast microtubules that were often stuck in persistent pausing states, rather than growing or shrinking as they did in normal cells. Based on how several mutations were likely to alter the topography of the β tubulin protein, the team predicted that microtubule interactions with other proteins might be interrupted. Among those potential disruptions were interactions with so-called kinesin motor proteins, which latch on to microtubules and ferry cargo in nerve cells. Indeed, using time-lapse imaging of microtubules in yeast cells, Tischfield showed that another subset of the mutations significantly diminished interactions with yeast kinesin motor proteins. These mutations all result in the degeneration of motor and sensory axon tracts in patients.

"What we saw in yeast was in agreement with the disease mouse model and showed that we could faithfully measure the properties of neuronal microtubules using this simpler organism," says Tischfield, noting that the overly stable mutant microtubules could indeed lack the fluidity necessary for proper axon growth and remodeling. "These studies provided us with a way to explain the kind of defects we saw in humans." They also provide a potential link to CFEOM1, an eye movement disorder closely related to CFEOM3 that the lab previously showed to result from mutations in a kinesin called KIF21A.

Engle believes that CFEOM and the TUBB3 syndromes can now serve as paradigms for human disorders of axon guidance. In addition, the team hopes that these findings will lead to improved care for affected individuals. “We can now provide genetic testing and counseling for affected individuals. And the more we understand the specific disorders and disease spectrum, the easier it will be – over time - to develop targeted therapies for these disorders,” Engle says.