# Researchers tease apart workings of a common gene

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Researchers at Weill Cornell Medical College have discovered why a tiny alteration in a brain gene, found in 20 percent of the population, contributes to the risk for anxiety, depression and memory loss.

Their discovery, reported in *Nature Communications*, describes new functions for the alteration, a single nucleotide polymorphism (SNP) in the brain-derived neurotrophic factor (BDNF) gene. This gene is a powerful regulator of the growth and function of neurons, and the establishment of brain circuitry. The common alteration occurs when a single "letter" of BDNF's genetic code is "misspelled."

The team of investigators, led by Dr. Clay Bracken, associate research professor of biochemistry and director of the nuclear magnetic resonance facility, Dr. Barbara Hempstead, professor of medicine, and Dr. Francis Lee, professor of psychiatry, all at Weill Cornell Medical College, discovered that the alteration appears to induce shrinkage of neurons from the hippocampus (an important region for memory and emotion), reducing connectivity between brain cells.

The discovery upends the prevailing theory about how the BDNF SNP alters the function of the brain, says Dr. Agustin Anastasia, first author of the article and a postdoctoral fellow in the Hempstead lab. "Research on BDNF is very active worldwide, and the conventional wisdom of the field was that the SNP reduced the amount of BDNF that was secreted. Therefore, many investigators were trying to increase production of the protein—but this effort was only moderately successful."

"While the SNP does decrease the amount of BDNF in neurons, it generates a protein, the Met66 prodomain, that is different from the Val66 prodomain that is generated by the 80 percent of the human population that does not carry the SNP," Dr. Hempstead says. "The Met66 prodomain binds to specific proteins on the surface of neurons, to induce the pruning or shrinkage of these neurons."

The findings offer mechanistic insight into why some depression and anxiety runs in families, Dr. Lee says. "There can be a heritable component to these diseases and it makes sense that a common variant in a gene could be involved," he says. "Just like hypertension contributes to the risk for heart disease, the BDNF alteration increases the risk of depression, anxiety and memory disorders—but is not the sole reason why they occur."

Still, targeted treatment for the genetic alteration could provide the first true benefit for affected patients, who often don't respond to traditional treatments, Dr. Lee says. "We can easily test patients for the mutation by using a simple blood test," he says. "We just need novel targeted treatments that alter the effects of the BDNF SNP— and now we have a good lead on what that therapy should do."

## The other half of the story

In 2006, Dr. Lee discovered that neuronal secretion of mutated BDNF was reduced, compared to secretion of wild-type BDNF, and generated a mouse that expressed the human BDNF SNP. That study appeared in *Science*. "It turns out we were only half right," Dr. Lee says. "This current study tells the rest of the story."

In the new study, the researchers used a combination of approaches to understand what the Met66 prodomain, generated by the BDNF SNP, was doing. Dr. Bracken led the structural biology work that defined the

alterations in the protein that were conferred by the BDNF SNP. The team also included Drs. Katrin Deinhardt and Moses Chao, BDNF biologists and investigators from the Skirball Institute at New York University School of Medicine, who used techniques to evaluate neuronal pruning.

The team knew that BDNF is manufactured inside neurons. One part of the protein, the prodomain, was known to help guide BDNF to the surface of neurons. BDNF released from cells stimulates the growth and activity of neighboring neurons. However, little was known about the prodomain itself; it was considered a useless or inactive protein.

The researchers used a variety of methods to study what actually happened to the prodomain with both the altered BDNF (Met66 prodomain), and in wild-type BDNF (Val66 prodomain).

They developed Met66-expressing mice, which displayed many of the detrimental effects (such as anxiety and alterations in memory formation) observed in human Met66 carriers, as well as tests to identify the activity of the Met66 prodomain. The researchers used advanced nuclear magnetic resonance analysis to identify the structure of the Met66 and Val66 prodomains and their interactions, Dr. Bracken says. Human cell lines were used to define the differences in prodomain binding and identify proteins and pathways critical for pruning of neurons.

"This was an exciting collaboration," Dr. Bracken says. "A lot of research studies focus on animal models of human disease, or on biophysics, or on the biology of neurons. We combined all three investigations, which was a very powerful approach because it utilized different ways of thinking about a common problem."

They discovered what they had long suspected but had not previously

been able to prove—that the Met66 prodomain was not an inert protein, but a degenerative agent. The team found that Met66 binds and activates a protein complex (SorCS2 and p75) known to shrink neurons, reducing their ability to communicate with neighboring neurons. They also discovered that the more common Val66 prodomain did not induce neuronal shrinkage.

"This was an unexpected but very exciting result. This study describes how a single substitution in BDNF causes a structural change in Met66 prodomain to endow it with a biological function," Dr. Hempstead says.

"The brain isn't set in stone—it wants to be able to build and rebuild, and in order to rebuild, it needs to break down neurons first. I suspect that Met66 prodomain is involved in a normal breaking-down process that involves SorC2 and p75, but in the altered BDNF, the balance is shifted in the wrong way," Dr. Lee says.

Using their Met66 mouse model, the researchers are now examining precisely what the mutation does to neurons—"how it alters the size and length of synapses or changes the way the synapses function," Dr. Anastasia says.

And, with this Met66 mouse model, they can examine drugs that could potentially target Met66 or block the proteins it binds to. "At the end of the day, understanding how the protein is made and how it acts is the goal. This will give us insights into how we can modify the activity of the Met66 prodomain, to help patients with this alteration in BDNF who suffer from anxiety or depression," Dr. Hempstead says.

Provided by Weill Cornell Medical College