New theory of Down syndrome cause may lead to new therapies

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Conventional wisdom among scientists for years has suggested that because individuals with Down syndrome have an extra chromosome, the disorder most likely results from the presence of too many genes or proteins contained in that additional structure.

But a recent study reveals that just the opposite could be true - that a deficiency of a protein in the brain of Down syndrome patients could contribute to the cognitive impairment and congenital heart defects that characterize the syndrome.

Scientists have shown in a series of experiments that there are lower levels of this protein in the brains of humans and mice with Down syndrome than are present in humans and mice without the disorder.

The researchers also showed that manually manipulating pieces of RNA that regulate the protein could increase protein levels in both human cell lines and mouse brains. In fact, an experimental drug that acts on those RNA segments returned this protein to normal levels in mice that model the syndrome.

When this RNA segment is overexpressed - meaning that more of it is present than needed in a cell - the protein level goes down, or is underexpressed. A total of at least five of these RNA segments are naturally overexpressed in persons with Down syndrome because the segments are housed on chromosome 21 - the chromosome that causes the disorder.

"We're talking about a paradigm-shifting idea that maybe we should look for underexpressed proteins and not overexpressed proteins in Down syndrome," said Terry Elton, senior author of the study and a professor of pharmacology at Ohio State University.

"What this offers to the Down syndrome community is the potential for at least five new therapeutic targets to pursue."

The Centers for Disease Control and Prevention estimates that about 13 of every 10,000 babies born in the United States each year have Down syndrome, characterized primarily by a mild-to-moderate range of intellectual disabilities, possible delayed language development and difficulties with physical coordination.

The study is published in a recent issue of the *Journal of Biological Chemistry*.

Elton, also interim director of Ohio State's Davis Heart and Lung Research Institute, stumbled upon this theory about Down syndrome while working on a different protein associated with cardiovascular disease. It turns out the protein he has studied for 25 years was regulated by one of these microRNAs that is known to be housed on chromosome 21.

A key role of RNA in a cell is to make protein, and proteins are the building blocks of all life. But the process has many steps. MicroRNAs are small pieces of RNA that bind to messenger RNA, which contains the actual set of instructions for building proteins. When that connection is made, however, the microRNA inhibits the building of the protein. Why that occurs is not completely understood, but increasingly microRNAs are considered tiny molecules that have a big impact in a number of physiological processes.

For his cardiovascular disease research, Elton found that a genetic trait in some people caused one specific microRNA to be bad at its job, leading to high protein levels that contribute to cardiovascular disease. This malfunctioning molecule is called microRNA-155, or miR-155.

"So we became interested in miR-155, and it is on chromosome 21. That's how we jumped to Down syndrome," Elton said.

There is also a strong link between the heart and Down syndrome. About half of those with the syndrome are born with congenital heart defects - problems with the heart's anatomy, not coronary arteries. But they do not experience cardiovascular disease or high blood pressure.

The advent of biomedical informatics has allowed scientists to use supercomputers to explore the human genome in a search for genes and their various relationships in the context of human disease. Elton consulted a bioinformatic database and found that five microRNAs sit on chromosome 21, and he and colleagues demonstrated in previous research that all five of them are overexpressed in the tissues, brains and hearts of Down syndrome patients.

"That means that whatever proteins these microRNAs work with are underexpressed," Elton said.

Further database exploration suggested that these five microRNAs target 1,695 proteins, all of which could cause problems in Down syndrome because they are underexpressed. To narrow that to a more manageable number, Elton's group had to make an educated guess based on a variety of data, including which proteins that are connected to these microRNAs are made by cells in the brain and heart - two areas most commonly affected by Down syndrome.

A protein surfaced as an attractive target to study: methyl-CpG-binding

protein 2, known as MeCP2. Among the reasons it seemed important: A mutation in this protein is already known to lead to Rett syndrome, a cognitive disorder.

"So we thought that it was more than a coincidence that this protein plays a role in normal brain development, and if the protein doesn't function right, you're going to have cognitive impairment. Maybe this is the connection," Elton said. "We still don't know if this is the most important protein related to Down syndrome. But we were able to go on and prove scientifically that MeCP2 is a target of these microRNAs on chromosome 21."

The researchers used just two of the five microRNAs on chromosome 21 for the experiments in this study, miR-155 and miR-802, to match the only microRNAs available in the genetically engineered mouse model of Down syndrome.

First, the researchers made copies of the relevant microRNAs. In human brain cell lines, they manipulated levels of those two molecules to show the inverse relationship with the protein. If the microRNAs were more active, the level of the MeCP2 protein went down. When the microRNAs were underexpressed, the protein levels went up.

Next, the researchers examined adult and fetal human brain tissue from healthy and Down syndrome samples obtained from a national tissue bank.

"In both adult and fetal Down syndrome brain samples, it didn't matter which area of the brain we were looking at, the MeCP2 proteins were down. These are just observations with no manipulation on our part, and the MeCP2 is almost non-existent in the Down syndrome brain," Elton said. "We marked the protein with a fluorescent molecule, and by comparison, we could visualize and appreciate how much MeCP2 was

being made by neurons in the control samples."

MeCP2 is a transcription factor, meaning it turns genes on and off. If its levels are too low in the brain, this suggests that genes influenced by its presence should be malfunctioning, too. Based on previous research by another group, Elton and colleagues focused on two genes affected by the MeCP2 protein for their next set of experiments.

Looking again at the human brain tissue samples, they found that the genes were indeed affected by the lowered protein level in Down syndrome brains - one gene that MeCP2 normally silences was in abundance, and the gene that should have been activated was underexpressed. Because the two genes examined have known roles in neural development, Elton said the results suggested even more strongly that the lowered protein's effects on the genes likely contribute to cognitive problems associated with Down syndrome.

Finally, the researchers tested an experimental drug called an antagomir on mice that serve as models for Down syndrome research. Antagomirs are relatively new agents that render microRNAs inactive. The scientists injected an antagomir into the brains of these mice to silence the miR-155 with the intent to increase levels of the MeCP2 protein. Seven days after the injection, the level of the protein in the treated mouse brains resembled levels in normal mouse brains.

"We showed that we can fix the protein abnormality in mice that model Down syndrome. But we can't undo the pathology that has already occurred," Elton said. "It's a starting point, but it appears that we have new therapeutic targets to consider."

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