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Scientists identify 'collateral vessel' gene that protects against stroke damage

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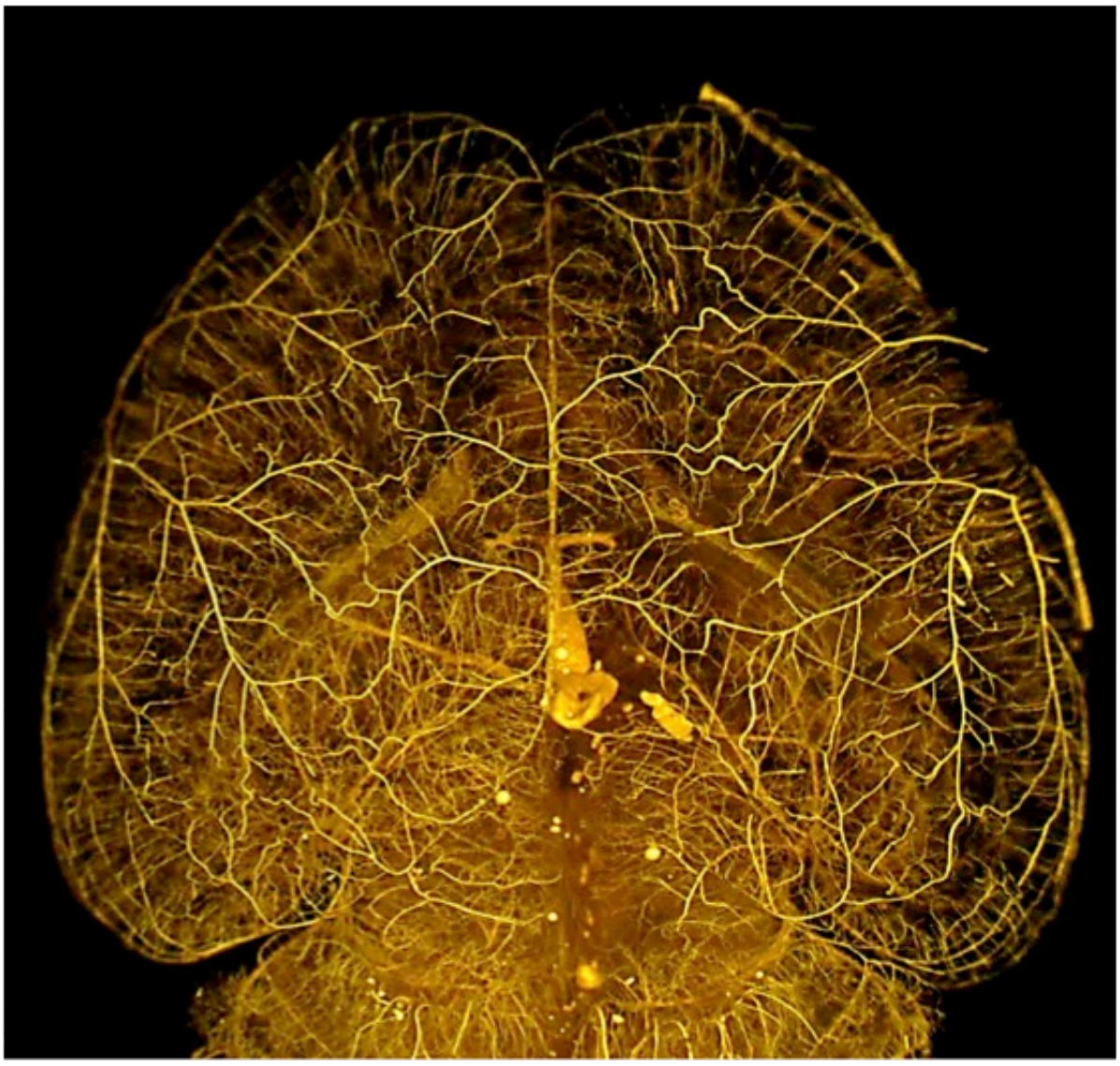

When the gene Rabep2 is deficient, the number and diameter of collateral blood vessels -- normally present during brain development -- are reduced by 50 to 60 percent, and the amount of brain tissue that dies after stroke is more than

doubled. Credit: Faber lab, UNC School of Medicine

Researchers at the University of North Carolina School of Medicine have found a major clue that may explain why some people sustain relatively little damage from strokes or heart attacks despite severe arterial blockages. The clue lies in the little-understood gene Rabep2.

Scientists have known that when an artery is blocked, the damage to tissues downstream is often limited because these tissues continue to be nourished by special "collateral" vessels that connect the tissue to other arteries. However, for reasons that haven't been understood, the number and size of these collateral vessels - and thus the protection they afford - can vary greatly from one individual to the next. The UNC scientists have now implicated the Rabep2 gene as a major contributor to this variation in collateral vessel formation.

In a paper published in the journal *Stroke*, a research team led by James Faber, PhD, professor of cell biology and physiology, found that variants of this gene account for most of the differences in collateral vasculature among laboratory mice. Faber said that since humans and mice are more than 90 percent genetically similar, the human version of Rabep2 is likely to have a comparable function.

Through a series of experiments, researchers replaced a defective variant of the gene in a mouse strain with poor collaterals with a normal copy of the gene, resulting in the formation of abundant collateral vessels during embryonic development and much greater resistance to tissue injury and cell death when the mice were subjected to experimental stroke as adults.

The UNC scientists hope that one day doctors will be able to use a

simple blood test to detect variants of the human form of the gene. This would help doctors quickly gauge the extent of collateral vessels in patients who experience heart attacks, strokes, peripheral artery disease, and occlusive disorders in other tissues.

"Whether patients have good or poor collaterals strongly influences the severity of tissue injury after an occlusion and affects doctors' decisions about how to treat patients or prescribe preventive measures," said Faber, who is also a member of the McAllister Heart Institute and the Curriculum in Neurobiology at UNC.

In principle, Faber added, the findings also could help lead to therapies that stimulate the formation of more collateral vessels in healthy people to reduce the severity of tissue injury in the event of a future arterial blockage, as well as in people who already have occlusions, thereby reducing damage and improving their recovery.

The *Stroke* paper comes nine years after Faber and his colleagues first observed that the extent of the collateral vasculature - and thus the damage after arterial occlusion - can differ greatly between different strains of lab mice, even though no differences in the rest of the circulatory systems were evident.

After this initial observation, Faber hypothesized that genetic differences might trigger these wide disparities in the extent of collaterals. Faber and colleagues began a tedious and lengthy search for the genetic factors responsible. They focused on collateral vessels in the brain, which are easier to image than in other tissues, and undertook experiments involving thousands of mice. By 2014, the group had narrowed the search to a small region on mouse chromosome 7, the variations of which accounted for nearly all of the differences in collateral development and tissue injury in the brains, hind limbs, and other tissues they examined.

In the new study, the researchers set out to identify the particular gene in this region that might explain the differences in collateral vessel development. From the 28 protein-coding genes in the region, the scientists were able to exclude 13, after determining that mice lacking any of those genes didn't have more or fewer collaterals.

Of the 15 remaining genes under suspicion, Faber and colleagues decided to focus on their top suspect, Rabep2. Little was known about this gene, but the scientists had previously found a Rabep2 variant in mouse strains with low collateral extent, whereas high-collateral strains had the normal version of the gene. The variant differs from the normal gene in only a single DNA "letter," but that change - because of its location - is predicted to impair the function of the resulting protein, Faber said.

Using new CRISPR gene-editing technology, the team was able to test the effect of this Rabep2 variant. They replaced the DNA letter in normal Rabep2 that is present in the genomes of high-collateral mice with the suspect variant. The result: the mice formed many fewer collaterals during development and had much greater stroke damage as adults. And this shift was even greater when the gene was deleted entirely.

Conversely, in mice from the low-collateral strain, replacing the variant gene with the normal one induced the animals to develop the abundant collateral vasculature present in the high-collateral strain. These beneficially "edited" mice were thus far more resistant to damage from stroke.

"We basically took mice of a strain that normally shows a very large area of tissue damage after an arterial obstruction in the brain, and - by editing that one gene - created mice that experienced much less damage after obstruction at the same site," Faber said.

How the Rabep2 protein influences the formation of collateral vessels in early life isn't entirely clear, but the gene is known to play a role in the transport and recycling of signaling molecules in cells. Faber and colleagues were able to find evidence suggesting that the lack of fully functional Rabep2 protein leads to deficient signaling of a major vascular growth factor, specifically in the cells that develop into collateral vessels.

Why does a deficiency of Rabep2 affect the development only of collateral vessels? Faber suspects that this is because the rest of the circulatory system's components - arteries, capillaries, and veins - are more important to survival thus have evolved extensive redundancies in their developmental pathways.

"An animal can have no collateral vessels and still be perfectly healthy," he said. "It doesn't really need those collaterals until it has an arterial occlusion."

Faber and his colleagues have now begun studies in patients with stroke to test for involvement of variants of Rabep2 and other related genes.

Provided by University of North Carolina Health Care