Faulty gene makes the brain too big—or too small

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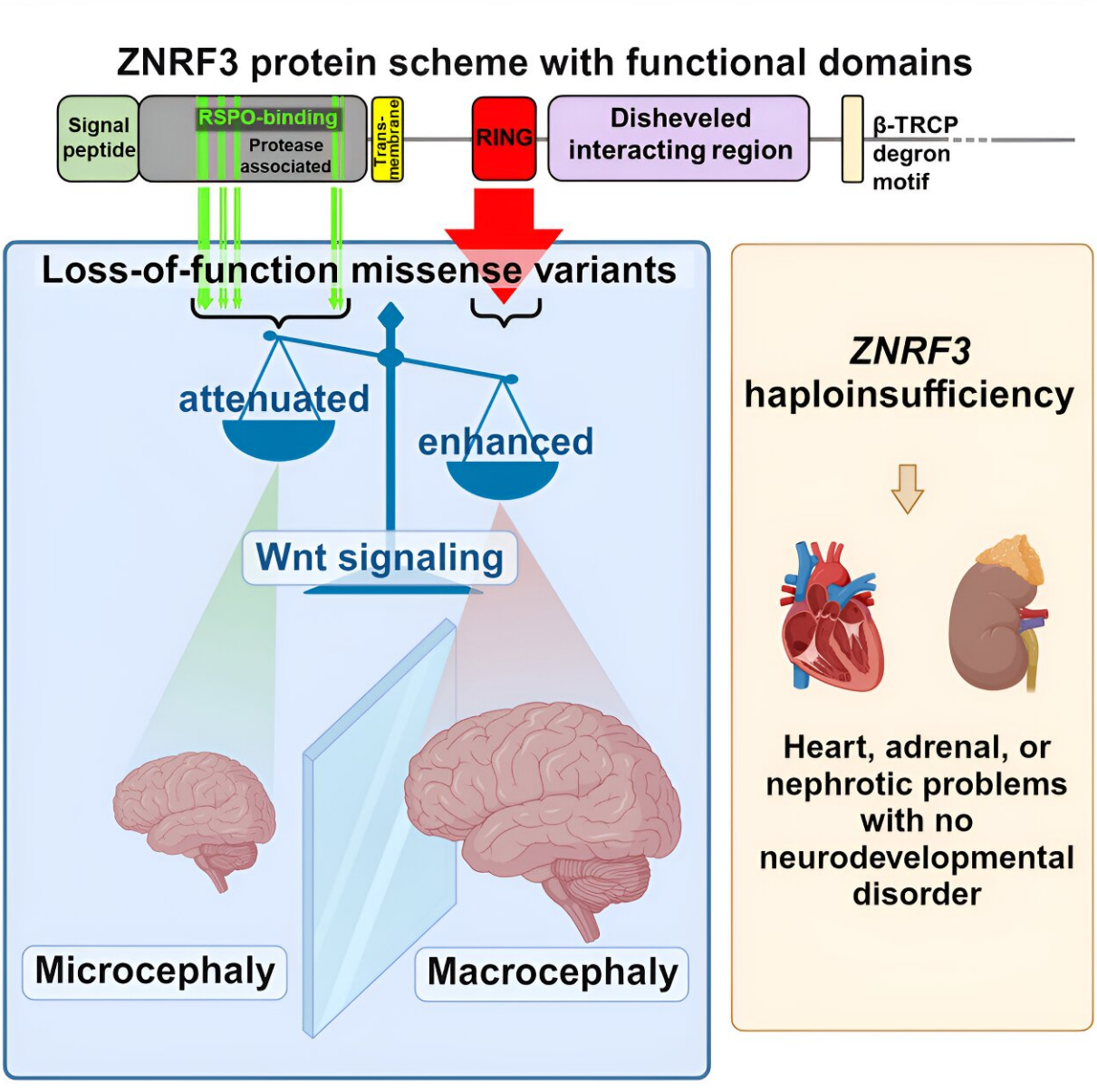

Credit: *The American Journal of Human Genetics* (2024). DOI: 10.1016/j.ajhg.2024.07.016

A gene called ZNRF3, known to be involved in cancer, also messes with the mind. The human brain relies on two copies of this gene to build a correctly sized brain. If one of the copies is defective, the brain will be either too small or too large—known as mirror effect—leading to various neurological symptoms.

Almost a decade ago, we saw a patient suffering from a very rare condition with an abnormally small brain, speech delay and ectodermal dysplasia—an inborn condition that affects hair, nails, teeth and skin. We sequenced part of her DNA and found a defect in one copy of the gene ZNRF3, a gene that was not yet associated with inborn disorders. This defect leads to the production of a harmful protein. So, we suspected that this was the cause.

Since then, we have collected DNA from 11 other patients from around the world who are believed to have a harmful mutation in the same gene. Most of them had a defective copy of ZNRF3 and showed variable neurodevelopmental signs with an abnormally large brain.

We tested the faulty versions of the gene in the lab and found a correlation between patients' brain size and the location of the mutations in the gene. After a long diagnostic odyssey, we were finally able to establish a definitive cause for the disease of these patients. Our study is published in the *American Journal of Human Genetics*.

Global collaboration enables research on rare conditions

As the condition described here is extremely rare, we relied on global collaboration through professional networking databases where we posted our candidate gene and received matches from around the world. We were able to collect a total of 11 additional patients with suspicious alterations in the same gene. Eight of them had one faulty copy of ZNRF3, while four patients had lost one copy.

Of the eight patients with defective copies, seven showed variable neurodevelopmental problems with an abnormally large brain, while one exhibited profound developmental delay with an abnormally small brain.

The four patients with only one functional copy showed no neurological symptoms, but malfunctions in other organs, such as the heart, adrenal gland or kidney. We did not observe any patient who had lost both copies, suggesting that absence of this gene is incompatible with life.

ZNRF3 is often mutated in several cancers

The gene ZNRF3 produces two copies of a protein that prevents the brain from making too many or too few brain cells. It also does the same in many other organs, so that mutations in its DNA sequence can lead to uncontrolled cell proliferation and are therefore associated with a variety of tumors, such as colon or adrenal cancer.

One of our analyses revealed that there is a small region of the ZNRF3 gene, called RING, where many mutations found in cancers are located compared to the rest of the gene. In fact, most of the patients with abnormally large brains have their mutations in the RING region. This means that they may have an increased risk of developing tumors during their lifetime.

Two regions in the ZNRF3 gene are critical for brain

size

Our analyses showed that almost all the mutations that lead to abnormal development are located in two distinct regions of the gene: one in the RING region, and the other in a smaller region that is important for interacting with another gene called RSPO. It turned out that almost all the defects in the RING region were from the patients with an abnormally large brain, while the defect in the RSPO-interacting region came from the patient with an abnormally small brain.

However, one patient had a fault in the RING region but had an abnormally small brain. We traced his family history and found that his mother used drugs heavily during her pregnancy, which could explain his abnormally small—instead of large—brain. Apparently, environmental influences can override genetic defects in this condition.

Lab experiments and modeling explain molecular defects

The gene ZNRF3 orchestrates the perfect balance of biochemical signals, particularly in the Wnt signaling pathway, needed to produce the right number of brain cells. This gene works in concert with the gene RSPO, which also interacts with the Wnt signaling.

In the lab, we created different defective versions of the ZNRF3 gene and measured the signal that represents changes in the Wnt signaling pathway. We found that the faults in the RING region (from the patients with abnormally large brains) increased the Wnt signaling, while the mutations in the RSPO-interacting region (from the patients with abnormally small brains) decreased the Wnt signaling.

These results showed that the right brain size depends on a balanced Wnt

signaling, which, once tipped toward too much or too little, can cause the brain to become too large or too small.

Sophisticated modeling of the defective versions of the ZNRF3 protein also revealed disrupted enzyme functions for the defects in the RING region, or impaired binding to the interacting protein RSPO for the defects in the RSPO-interacting region.

Better monitoring and treatment of patients due to cancer risk

As FDA-approved modulators of the Wnt signaling pathway are available, these findings open the possibility of using Wnt modulators therapeutically. This intervention must, however, be approached with caution, as a Wnt inhibitor should only be considered for the patients with an abnormally large brain and not for those with an abnormally small brain, even if they have faulty copies of the same gene.

The ZNRF3 gene joins a list of tens of other genes that are involved in the Wnt signaling pathway that have been linked to brain size. Nonetheless, it is so far the only one of these genes to lead to opposing brain sizes with a distinct region-specific pattern, known as a mirror effect.

Since the Wnt signaling pathway is linked to cancer when disrupted, monitoring and intervention could be planned and personalized for patients with a faulty ZNRF3 gene.

More information: Paranchai Boonsawat et al, Deleterious ZNRF3 germline variants cause neurodevelopmental disorders with mirror brain phenotypes via domain-specific effects on Wnt/β-catenin signaling, *The American Journal of Human Genetics* (2024). DOI:

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