Basic science shows how a single mutation causes ataxia

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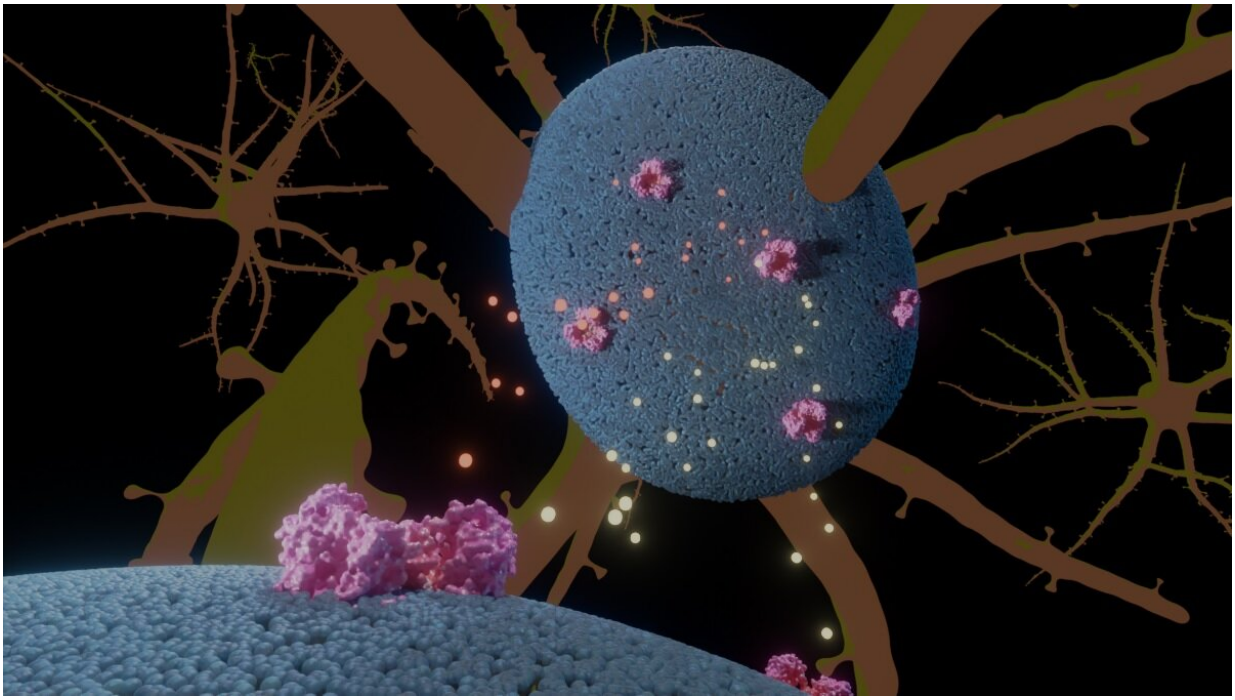

Illustration of the glutamate transporter (pink) in neural cells (blue), with glutamate and anions in yellow and orange. Credit: A. Guskov, University of Groningen

Worldwide, only a handful of patients are known to suffer from episodic ataxia type 6, a neurological disease that causes transient loss of muscle control. The cause lies in a mutation that changes a single amino acid in a protein that transports the neurotransmitter glutamate across the

membrane of neural cells. Researchers from the University of Groningen (the Netherlands) have elucidated how the mutation causes these cells to malfunction. Their results appear in *Nature Communications*.

Patients with ataxia lose control of their muscles, which can for example affect how they move or talk. An extremely rare form of this disease is episodic ataxia type 6 (EA6), in which patients suffer episodes of ataxia. Worldwide, there are just over a dozen known patients, including one family in the Netherlands. It is known that EA6 is caused by a single mutation, but how this mutation can have such a dramatic effect was thus far a mystery.

"This protein transports glutamate across the membrane of neural cells," explains structural biologist Albert Guskov. The protein is inserted in the cell membrane, and the mutation changes a proline amino acid in one of the helical transmembrane domains into an arginine.

"A proline in a helix typically causes a kink," explains Guskov. "If a proline is changed into an arginine, we would expect this kink to disappear. To test this, we studied the structure of the mutated protein."

Since the human transport protein is difficult to study in the lab, Guskov and his colleagues used an analogous protein from archaea, an ancient form of unicellular organism. "This archaeal protein has been well conserved throughout evolution, and we know from previous work that it is a good model for the human transport protein, even though it transports aspartate and not glutamate," explains Guskov.

Using cryo-electron microscopy on normal and mutated proteins placed in lipid nanodiscs, the team was able to compare the shape of the mutated protein to the normal version. In previous studies, the team had shown that part of the protein moves up and down through the

membrane, much like an elevator. The hypothesis was that the mutation would cause the transmembrane kink in the protein to disappear, and that this would change the protein's shape and block the elevator movement.

However, that was not the case. Gustov says, "To our surprise, the kink was still there." Nevertheless, the mutation did affect the functioning of the protein. "The transport rate was reduced by a factor of two, compared to the normal protein." Furthermore, during transport of the aspartate, the protein transiently formed an anion channel. "And in the mutated protein, ion transport was three times higher."

Somehow, the arginine that replaced the proline did not alter the shape of the transport protein, but it did affect its function. Therefore, the researchers performed molecular dynamics simulations, which show all the interactions of the amino acids of the protein with their surroundings. "What we noticed is that a salt bridge is formed between the arginine amino acid and the lipids of the membrane." This salt bridge, a form of attraction between molecules, appears to slow down the movement of the elevator part of the protein.

Gustov says, "If this elevator moves more slowly, it explains the decrease in aspartate transport, but it also means the transient ion channel remains open longer, thus enabling more anions to pass through." In human neural cells, this would lead to a reduced transport of the neurotransmitter glutamate, and increased anion imbalance. These findings explain how this mutation causes ataxia. "Both have very nasty consequences for the functioning of neural cells."

However, there is no simple way to remedy the effect of the mutation. Gustov says, "Furthermore, this transporter is present throughout the body, so any drug affecting it will probably have serious side effects." Also, since there are only a handful of patients, no drug company would

invest in a cure. "Although there might be a lot more patients. Since it is an episodic illness and the symptoms can be mild, many people might not be aware of it. They are simply used to feeling unwell for a few days at a time, just like someone who suffers from migraine."

For the scientific community, these findings raise a number of intriguing questions. Gustov says, "The protein has been very well conserved throughout evolutionary history. So why did this transient anion channel appear, and has it turned out to be so beneficial for archaea that it was carried over time right to our own neurons? That is what we would like to understand."

More information: Emanuela Colucci et al, Mutation in glutamate transporter homologue GltTk provides insights into pathologic mechanism of episodic ataxia 6, *Nature Communications* (2023). DOI: 10.1038/s41467-023-37503-y

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