JGP Journal of General Physiology

RESEARCH NEWS

ATP1A1 variants spring a leak

Ben Short[1]

JGP study (Colmano et al. https://doi.org/10.1085/jgp.202513959) identifies passive leak currents as a likely common mechanism underlying hypomagnesemia with treatment-resistant seizures in patients with certain mutations in the Na^+, K^+-ATPase α1 subunit.

Germline mutations in *ATP1A1*, which encodes the ubiquitously expressed, catalytic α1 subunit of the Na^+, K^+-ATPase (NKA), cause a variety of diseases affecting endocrine and renal tissues, as well as the central and peripheral nervous systems (1). But it remains unclear why particular *ATP1A1* variants cause a disease affecting one tissue, while other variants cause a different disease in another. In this issue of *JGP*, Colmano et al. show that four variants associated with hypomagnesemia with treatment-resistant seizures all display passive leak currents that can likely explain both of these disease-specific symptoms (2).

Nicolas Colmano, Daniel Self, and Pablo Artigas.

Charcot-Marie-Tooth (CMT) neuropathies are the most common disease associated with *ATP1A1* mutations. These variants reduce NKA function but, for some reason, they only affect the peripheral nervous system. In contrast, four *ATP1A1* variants have been identified in pediatric patients with hypomagnesemia accompanied by cognitive delay and seizures (3, 4). NKA is critical for Mg^{2+} reabsorption by the kidneys, and low levels of Mg^{2+} in the blood can cause convulsions. But these particular patients still experience seizures after Mg^{2+} supplementation, indicating that these variants also cause a separate issue in the CNS.

The four variants all introduce an arginine residue into the middle of one of NKA's transmembrane domains. Previous studies showed that, like the mutations causing CMT, these arginine substitutions reduce NKA function (3, 4). Some, but not all, of the variants also display passive leak currents that could have a dominant-negative effect in cells. "Why do these four mutations cause hypomagnesemia with treatment-resistant seizures, rather than CMT?" says Pablo Artigas, a professor at Texas Tech University Health Sciences Center. "We wanted to study all four variants in a more detailed way under the same conditions."

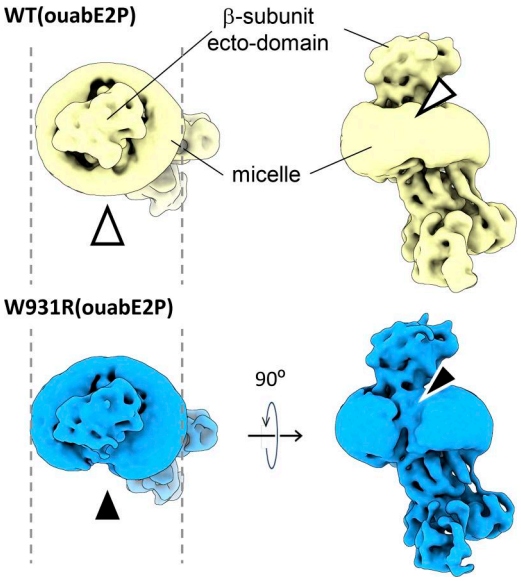
Artigas and colleagues, led by postdoc Nicolas Colmano and graduate student Daniel Self, first confirmed that all four variants have reduced function in cells. In addition to previously reported reductions in ion transport rates, Colmano et al. found that decreased expression at the cell surface may contribute to this loss of function. But a reduction in NKA function does not distinguish these variants from *ATP1A1* mutations causing CMT, so the researchers next expressed the variants in *Xenopus* oocytes to examine their electrophysiology.

Wild-type NKA displays a robust K^+-activated outward current, reflecting the pump's expulsion of three Na^+ in exchange for two K^+ ions. Two of the *ATP1A1* variants—L302R and G303R—showed almost no current in response to K^+, but the other two mutants—M859R and W931R—produced reduced outward currents in comparison with wild-type pumps, reflecting their partial retention of NKA activity.

In contrast to previous reports, however, all four variants displayed passive, inward leak currents at physiological membrane potentials that, at least in the case of G303R and W931R, reflect the permeation of both Na^+ and Cl^-. Because these currents are common to the variants causing hypomagnesemia with treatment-resistant seizures, it likely underlies the symptoms experienced by patients with these mutations. Certainly, leaky permeation of Na^+ and/or Cl^- would depolarize patient neurons, leaving them hyperexcitable and prone to seizures. Mg^{2+} reabsorption in the distal convoluted tubule of the kidney is highly sensitive to membrane potential and the Cl^- intracellular concentration as well, suggesting that abnormal leak currents could

[1]Science Writer, Rockefeller University Press, New York, NY, USA.

Correspondence to Ben Short: bshort@rockefeller.edu.



A comparison of the cryo-EM structures of wild-type (top) and W931R (bottom) NKA shows that the surrounding micelle is distorted (black arrowhead) in the vicinity of the mutated residue. This distortion could create a pathway for ion permeation, giving rise to the leak current observed in W931R and all *ATP1A1* variants associated with hypomagnesemia with treatment-resistant seizures.

also disrupt renal function and cause hypomagnesemia.

Colmano et al. looked at the W931R variant in more detail. Enzymatic studies revealed that, in keeping with its electrophysiology, the W931R mutant can actively exchange Na^+ and K^+. But one of its Na^+-binding sites has a reduced affinity for Na^+, decreasing overall transport rates. In collaboration with Kazuhiro Abe's group at Hokkaido University, the researchers obtained cryo-EM structures of the W931R variant in detergent micelles. The mutation, which is in the M8 transmembrane segment of the α1 subunit, disrupts a hydrogen bond network, destabilizing the Na^+-binding site that has a reduced affinity for this cation, and causes a kink in the adjacent M9 segment in one of the structures solved.

The structures also suggested how the mutation might give rise to a leak current. The detergent micelle appeared to be distorted in the region surrounding the mutant arginine residue, potentially disrupting the protein–lipid interface. "We speculate that the leak current happens through a distortion of the plasma membrane in this protein/lipid interface region," says Artigas.

The other *ATP1A1* mutations underlying hypomagnesemia with treatment-resistant seizures could cause similar membrane distortions around a mutant arginine residue. To test the idea that this creates a pathway for ion leakage, Artigas and colleagues hope to obtain structures of the disease variants embedded in nanodiscs, which more accurately reflect the organization of the lipid bilayer.

References

1. Biondo, E.D., et al. 2021. *Am. J. Physiol. Cell Physiol.* https://doi.org/10.1152/ajpcell.00059.2021
2. Colmano, N., et al. 2026. *J. Gen. Physiol.* https://doi.org/10.1085/jgp.202513959
3. Schlingmann, K.P, et al. 2018. *Am. J. Hum. Genet.* https://doi.org/10.1016/j.ajhg.2018.10.004
4. Ygberg, S., et al. 2021. *J. Biol. Chem.* https://doi.org/10.1016/j.jbc.2021.101355