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A missense mutation makes a mess of Ca^{2+} sensing

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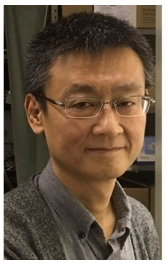
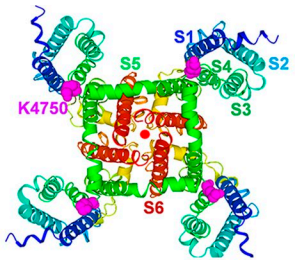
A new JGP study shows how a disease-causing mutation in RyR2 dramatically alters channel behavior.



Co–first authors Akira Uehara (left) and Takashi Murayama (middle) and senior author Nagomi Kurebayashi (right), together with co–first author Midori Yasukochi and others (not shown), demonstrate how a disease-causing mutation at lysine 4750 affects gating of human ryanodine receptor 2. Residue 4750 is located on the channel's cytoplasmic face, near its transmembrane pore (highlighted in pink in this top-down view ribbon diagram). PHOTOS COURTESY OF THE AUTHORS.

The human heart beats more than 2.5 billion times over the course of an average lifetime. The ryanodine receptor type 2 (RyR2) helps regulate these beats, and mutations in this ion channel cause catecholamine-induced polymorphic ventricular tachycardia (CPVT). Uehara et al. describe how one particular disease-causing mutation dramatically affects RyR2 behavior (1).

The arrival of a cardiac action potential allows extracellular Ca^{2+} to enter the cell and bind to RyR2 channels embedded in the membrane of the endoplasmic reticulum (called the sarcoplasmic reticulum, or SR, in muscle cells), causing the channels to open and release Ca^{2+} from the SR into the cytoplasm. The resultant rise in cytoplasmic Ca^{2+} activates the cardiomyocyte's actomyosin contractile apparatus and also jumpstarts mechanisms that pump Ca^{2+} back out of the cytoplasm to prepare the cell for the arrival of the next action potential.

Mutations in human RyR2 can allow excessive SR Ca^{2+} leak, provoking contractions out of sync with cardiac action potentials and causing CPVT patients to develop an abnormally rapid heartbeat in response to exercise or stress (2). A 2009 study (3) identified a young girl with a point mutation in one of her RyR2 alleles that replaced the lysine residue at amino acid 4750 with a glutamine residue. "The patient harboring this mutant exhibits the most severe clinical phenotype of CPVT reported thus far, which likely shows us a clear molecular dysfunction of the RyR2," says Dr. Akira Uehara, from Fukuoka University in Japan.

To investigate how the K4750Q mutation affects RyR2 behavior, Uehara et al. expressed recombinant RyR2 in HEK 293 or cardiac-derived HL-1 cells and monitored intracellular Ca^{2+} dynamics using a cytoplasmic Ca^{2+} sensor. Whereas cells expressing wild-type RyR2 occasionally experienced brief bursts of SR Ca^{2+} release and reuptake as rising external Ca^{2+} levels drove Ca^{2+} into the cytoplasm, cells expressing RyR2-K4750Q experienced these oscillations at lower external Ca^{2+} concentrations and with much greater frequency.

For a clearer view of how cells' internal calcium stores are handled by RyR2-K4750Q, Uehara and colleagues used a novel technique, imaging two Ca^{2+}-sensing dyes to simultaneously visualize calcium levels in both the ER lumen and the cytoplasm. The authors observed that mutant RyR2 is extremely leaky. "Our imaging experiment demonstrated that the lumenal Ca^{2+} concentration is significantly decreased by the K4750Q mutation," notes Uehara.

> "We were amazed at the dramatic alteration in all three RyR2 Ca^{2+} sensitivity mechanisms."

What explains the leakiness of the mutant channels? Recent physiological and modeling studies (4) suggest that Ca^{2+} governs RyR2's transmembrane pore in multiple ways. First, although its transmembrane pore is most often closed to ion traffic in the absence of Ca^{2+}, researchers think that RyR2 opens when Ca^{2+} binds to an activation site on the cytoplasmic face of the channel. Second, Ca^{2+} can also bind to another, lower-affinity site on RyR2 and destabilize the channel's open state. Therefore, RyR2 channels are mostly open at intermediate levels of cytoplasmic Ca^{2+} but mostly closed at very low or very high cytoplasmic levels. Finally, wild-type RyR2 can open in response to high SR lumenal Ca^{2+}, which can act via intra-SR mechanisms (5) and/or pass through the channel to activate neighboring RyR2s (6).

Uehara and colleagues found that the K4750Q mutation makes RyR2 much more sensitive to Ca^{2+} activation and simultaneously abolishes Ca^{2+} inactivation. Therefore, the channel opens at lower Ca^{2+} levels than wild type and remains open even at high Ca^{2+} levels. Single-channel studies confirmed that RyR2-K4750Q also opens at lower SR lumenal Ca^{2+} levels than do wild-type channels. "We were amazed at the dramatic alteration in all three RyR2 Ca^{2+} sensitivity mechanisms induced by this single missense point mutation," says Uehara.

Uehara et al. theorize that the lysine at 4750 may help stabilize RyR2's closed conformation by forming salt bridges with nearby charged amino acids—interactions that could be lost upon substitution of a neutral residue such as glutamine. Functional studies are already underway to test this, and Uehara invites collaborations on structural studies to learn more about how K4750Q affects RyR2 activity.

1. Uehara, A., et al. 2017. *J. Gen. Physiol.* http://dx.doi.org/10.1085/jgp.201611624
2. Priori, S.G., et al. 2001. *Circulation.* 103:196–200.
3. Sugiyasu, A., et al. 2009. *Pacing Clin. Electrophysiol.* 32:e21–e24.
4. Mukherjee, S., et al. 2012. *J. Gen. Physiol.* 140:139–158.
5. Chen, W., et al. 2014. *Nat. Med.* 20:184–192.
6. Tripathy, A., and G. Meissner. 1996. *Biophys. J.* 70:2600–2615.

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