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Structural insights into catecholaminergic polymorphic ventricular tachycardia-associated RyR2 mutant channels using a three-dimensional in silico model

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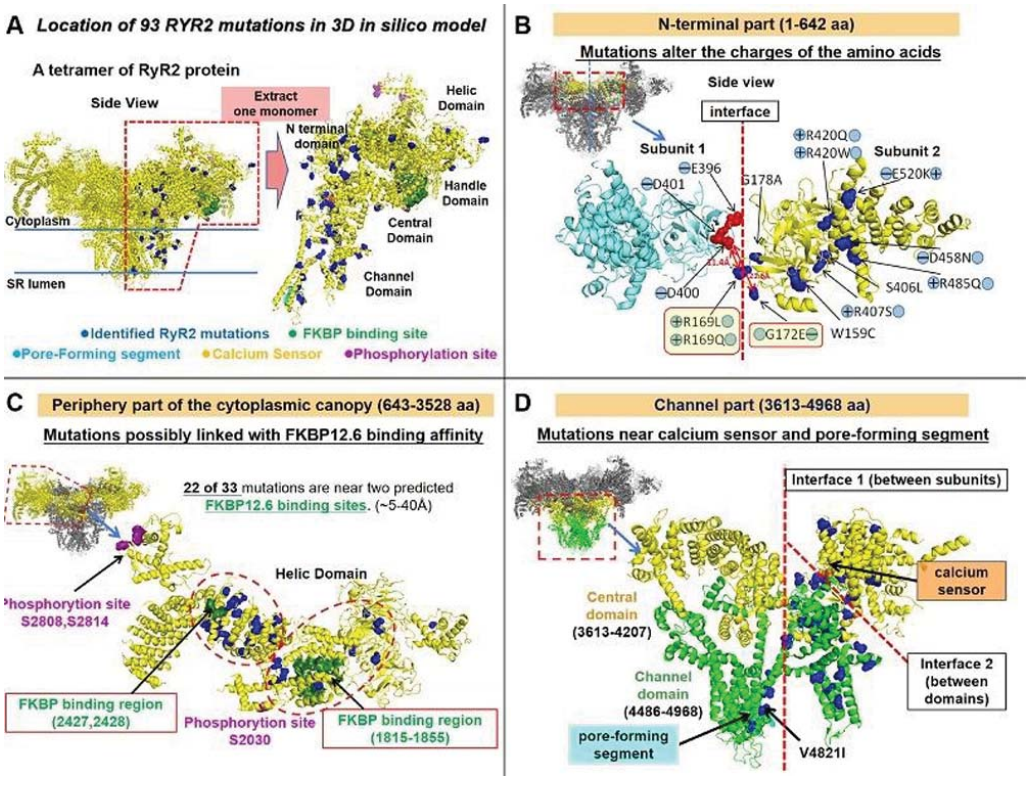
Background: The cardiac ryanodine receptors (RyR2) are large tetrameric calcium-permeant ion channels found in cardiac muscle sarcoplasmic reticulum, which play an important role in the control of intracellular Ca2+ release and cardiac contraction. Mutations in the RYR2 gene are associated with lethal arrhythmia diseases including catecholaminergic polymorphic ventricular tachycardia (CPVT) resulting from increased diastolic Ca2+ leak from mutant channels. RyR2 is a huge protein that each subunit of tetramer is comprised of 4967 amino acids, which hampers the detailed in vitro analysis of RyR2 mutant channels.

Purpose: We aimed to analyze the structural features of RyR2 mutant channels identified in our cohort with inherited arrhythmias using RyR2 three-dimensional (3D) in silico model to reveal the arrhythmogenic mechanisms.

Methods: A targeted next-generation sequencing panel for inherited arrhythmias was employed for genetic diagnosis of the patients. Then, we mapped the identified mutations on RyR2 3D structural model developed by cryo-EM images (PDB: 5go9, 5goa, Peng Science 2016) and investigated the relationship between the location of the mutations and specific functional sites.

Results: As a result of genetic analysis, we identified 93 RYR2 mutations from 112 probands with CPVT (n=93) or long-QT syndrome (LQTS) (n=19).64 of 93 (69%) RYR2 mutations are located in three "hot-spot" area (N-terminal (residues 77–466), central (2246–2534), and channel (3778–4959) hotspot. RyR2 3D in silico modeling revealed that the mutations are regionally distributed mainly in three parts: N-terminal, periphery, and channel part (Figure A). In N-terminal part (1–642 amino acid), 9 of 13 mutations alter the charges of the amino acids (Figure B). Especially, R169L, R169Q, and G172E are close to the interface between two neighboring subunits (~20Å). These mutations which change the amino acid charge may cause a complete disruption of the ionic pair network and result in largest structural changes, which facilitates RyR2 channel opening. In periphery part (643–3528aa), 22 of 33 mutations are close to the two predicted binding sites of FKBP12.6, a stabilizer of RyR2 (~5–40Å, Figure C). The mutations are supposed to disturb the binding affinity to the FKBP12.6 resulting in RyR2 channel instability. In channel part (3613–4968aa), 16 of 40 mutations are located near two interface. (FigureD) 12 mutations are close to the Ca2+ sensor and the other 4 mutations are adjacent to the pore-forming segment. Especially, V4821I is just located on this segment and strongly expected to impair the channel function. Above all, RyR2 3D in silico modeling revealed that 63 of all 93 (68%) identified mutations are supposed to be pathogenic.

Conclusion: 3D structural model of RyR2 is useful for the investigation of the pathogenic mechanisms of CPVT-related mutations. Further studies are needed to elucidate the relationship between the location of the mutations and clinical phenotypes.



Location of RYR2 mutations in 3D model