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RYR2 and Calcium Signalling: A Review

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ABSTRACT
Cardiac muscle function is heavily dependent on signalling pathways, which regulate contraction and relaxation in response to a variety of external stimuli. Ultimately, one of the cardiac muscle's core dependencies comes from the concentration of calcium within the sarcoplasmic reticulum microenvironment. In this review, we explore the functional significance of RYR2, one of the channels that contribute to the concentration of calcium inside cardiac myocytes as well as the implication of another cellular-level process that contribute to the contraction of the heart.

Keywords: RYR2; ATP; cardiac myocytes; cardiac muscle's;

1. Biological and Functional Significance of Other Proteins in Cardiac Contraction

In cardiac muscle, contraction happens through structured, intentional arrangement of two muscular proteins: actin and myosin. Actin is composed of globular subunits known as G actin, which then assemble into the filamentous F actin, an assembly that takes place to retain the structural polarity that was inherent in the G actin subunits (Figure I). Due to this structural polarity, there are plus and minus ends to actin filaments that guide the subsequent myosin movement [3]. Myosin, an essential motor protein within cardiac muscle, takes advantage of this polarity in its movement due to the inherent characteristic of being a plus-end directed motor [24].

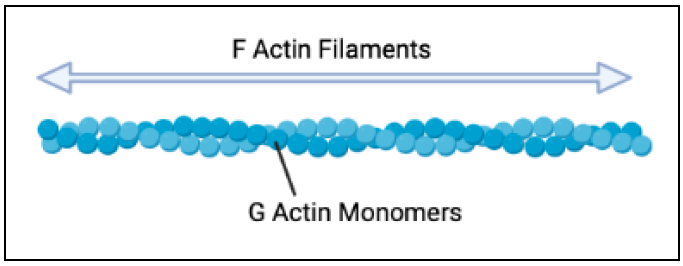

Fig1: G actin monomers assemble to form F actin filaments, maintaining structural polarity.

Movement is triggered by ATPase activity whereby the energy generated through the hydrolysis of the ATP allows for movement against the actin filament [11]. Myosin II, the subtype of myosin the acts intramuscularly, is composed of 2 heavy chains, both of which have a globular head and a filamentous tail domain, the tail domains intertwine while the head domains remain accessible to the actin [39] [2]. The movement of myosin heads upon the surface of actin filaments happens through the use of the myosin cross bridge, which relies uses the aforementioned ATPase in order to cleave a phosphate group from ATP. Myosin II is able to make stable connection with the actin as it moves due to the inherent structure in its head region – the head region of each myosin II head has two important binding pockets,

one which is specifically catered for ATP and one that is designed to bind actin [31]. The tail domain, on the other hand, are designed to bind specific substrates suited to the use of the particular function [9] [38] . Myosin II was also experimentally determined to be a plus-end directed motor by virtue of an experiment from the early 2000s that examined the result of adhering the myosin heads on a glass plate and adding actin filaments; after noting the minus end leading movement of the actin filaments, the plus end direction of myosin was concluded [20].

Within the context of the myosin cross bridge, this ATPase-dependent movement is triggered through the initial binding of ATP to the myosin head, currently attached to the actin filament [4]. The binding of ATP causes a conformational change within the myosin that leads the head to dissociate from the actin filament; following the binding of the nucleotide, it is quickly hydrolysed to leave behind the adenosine subunit and the myosin head pivots to binds a new actin filament subunit upstream of the original [12]. Since myosin is a plus-end directed motor, it moves toward the plus end of the actin filament. When ATP is hydrolysed, the two subunits that are the result of the hydrolysis (the ADP and the inorganic phosphate) are still attached to the myosin head. However, the release of the inorganic phosphate eventually leads to the head pivoting movement that moves the filament [4]. This pivoting and shifting movement is known as the 'power stroke' and is the direct cause of the movement of actin filament. Myosin II, the motor protein that moves cardiac actin filaments, has two heads – despite having two heads, myosin does not have processive proclivities and so the movement of each of the heads happens independent of each other [40].

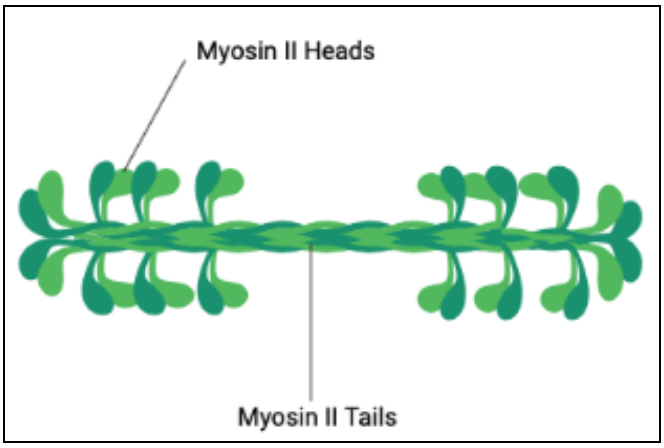

Fig2 : Myosin II tail domains combine to form bipolar thick filaments with heads at either side.

Within cardiac muscle, myosin II tail domains (composed of coiled coil) combine to form structures called bipolar thick filaments such that there are myosin heads at either end of the structure (Figure II). These filamentous forms of myosin are those that are frequently found in heart and contribute to contraction due their aforementioned ability to move along actin filaments [13][7].

The actin filaments arranged with great functional consequence; the plus ends are located on the outside of the sarcomere where the filament itself is anchored to the Z disk by a capping protein known as Cap Z [27].The Z disk serves as the border between sarcomeres and delineates between each. Myosin (in the bipolar thick format) are also organized as running across a sarcomere, parallel to and between actin filaments [28]. Due to their structure as bipolar thick filaments with heads protruding from either side of the myosin, the myosin heads are able to binds to actin filaments on both sides of the sarcomere [23] . Additionally, due to the fact that plus ends of the actin filament are anchored at the outsides of the

sarcomere and since myosin heads are plus end directed motors, myosin will move toward the plus ends (i.e., the Z disks and the outer ends of the sarcomere), effectively shortening it, while actin filaments and myosin filaments stay the same length as original [13] [5].

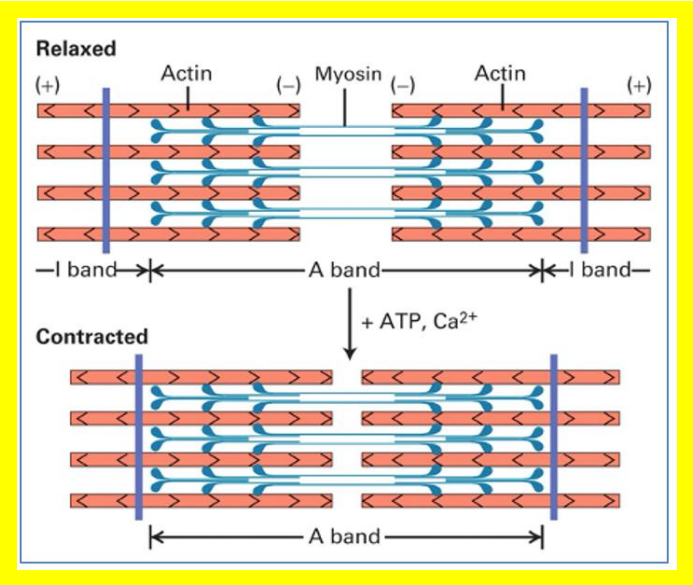

Fig 3: Relaxed and Contracted ATP

Given this, a likely conclusion is that the contraction of muscle is ATP-dependent, as it is the ATPase activity that drives the movement of the myosin and that movement ultimately drives the shortening of the sarcoplasmic reticulum – resulting in muscle contraction [7] . However, this is not the case as ATP in the muscles is not limiting, indicating that the cell does not control muscle contraction by presence or absence of ATP. Calcium, in fact, is the limiting reagent and controls muscle contraction, and is an important secondary messenger for several other signal transduction pathways throughout the body [19]. This dependence on calcium has to do with the inherent structure of cardiac muscle. Cardiac muscle is striated due its composure of individual myofibers, each of which are composed of individual myofibrils. Within the context of cardiac muscle, these myofibrils are surrounded by a variety of tubular structures [37]. One such tubular structure is known as a transverse tubule, or “T tubule,” while the other is the sarcoplasmic reticulum. Attached to the myofibrils are mitochondria, the site of metabolism and the source of ATP production that is necessary to fuel the movement of the myosin motors. The sarcoplasmic reticulum and the T tubules run perpendicular to one another, with the T tubules running along the Z lines (located at the site of the Z disks that delineate between each individual sarcomere). On the other hand, the sarcoplasmic reticulum runs essentially parallel to the myofibrils and are able to make contact with the mitochondria [34].

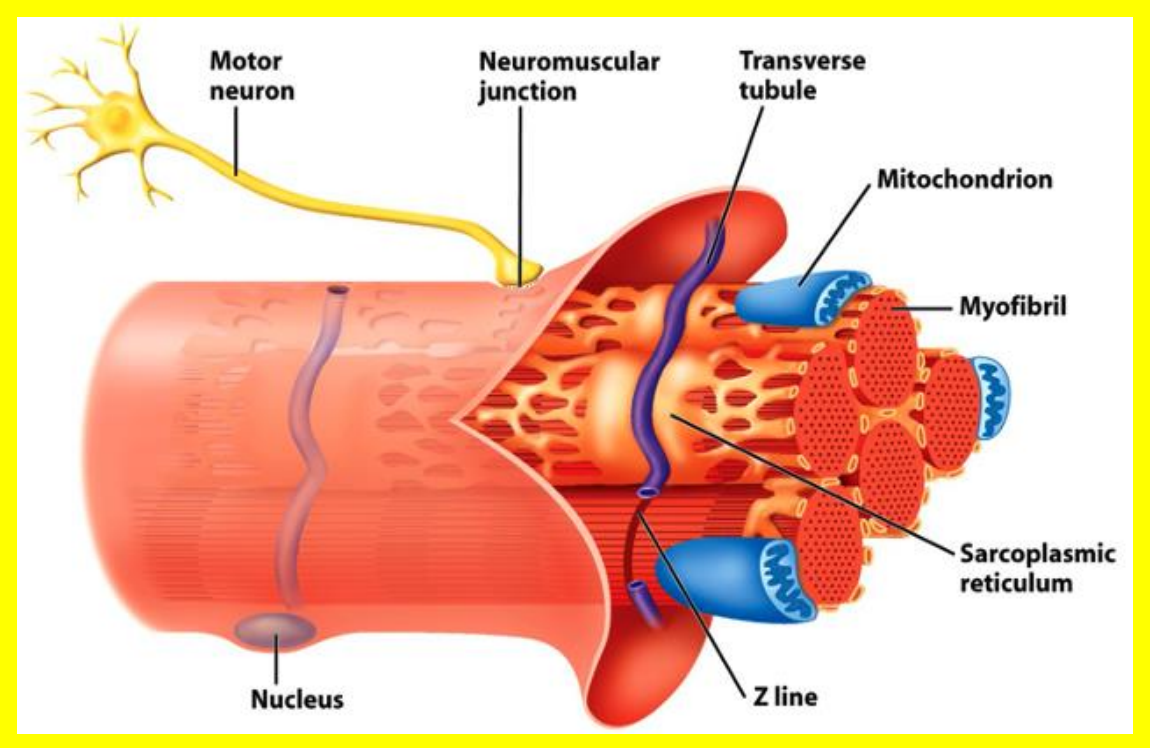

Fig 4: myofibrils

2. Calcium in Muscle Contraction

While the physical mechanics of cardiac contraction are driven by the combined action of motor protein myosin acting on the actin filaments throughout the length of the sarcoplasmic reticulum, the physical signal for the cardiac contraction comes from the neuronal release of acetylcholine [15]. Acetylcholine, once released from the neuron, binds to ligand-gated sodium channels that are inherent in the plasma membrane of cardiac myocytes – which in turn induces membranal depolarization [15] [38]. This depolarization spreads throughout the plasma membrane of the myocyte and ultimately moves into the transverse tubule that is attached to the plasma membrane. T tubules depolarization is not instantaneous due to the fact that T tubules are only present at the Z lines and there is additional spread of depolarization required for signal to reach the T tubules. T tubules invaginating the plasma membrane are also closely and perpendicularly associated with terminal cisternae of the sarcoplasmic reticulum. When the T tubule is depolarized, a protein in the T tubule voltage sensor dihydropyridine receptor, or DHP that is very sensitive to the depolarization and causes the stimulation of the closely related cardiac ryanodine receptor, RYR2 [32] [13]. RYR2 then releases calcium into the cytosol of the sarcoplasmic reticulum. This then stimulates additional movement of calcium from a variety of different channels including ATPase channels and sodium-calcium pumps, a collective action known as calcium-induced calcium release (CICR) [8] . DHP, a modulator of RYR2 also is responsible for release of calcium through coupling with T-tubules within the sarcoplasmic reticulum [10]. Other proteins within the ryanodine receptor protein family additionally mediate calcium release, as well as another protein family, the inositol 1,4,5-triphosphate receptors (IP_3Rs) [19].

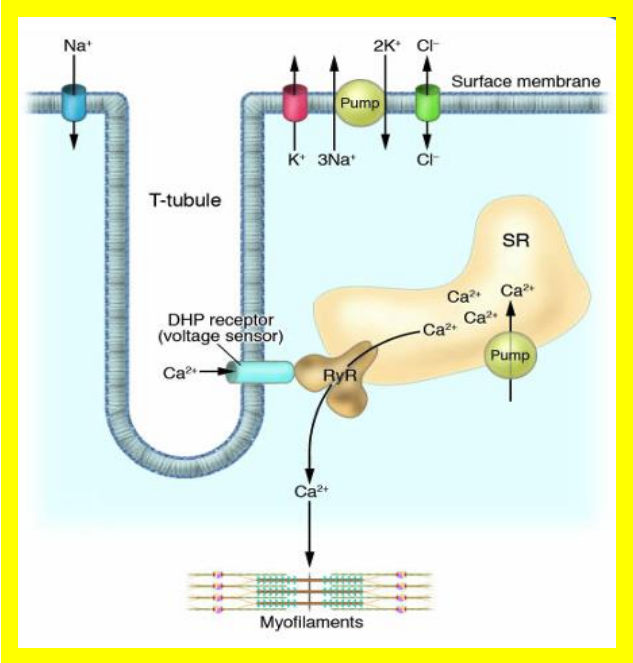

Fig 5: Myofilaments

Calcium is implicated specifically with two other proteins, troponin C and tropomyosin, which are involved in the regulation of the actin-myosin movement. Tropomyosin is consistently bound to the actin filament such that it blocks the interaction of the myosin heads with the actin filaments, effectively preventing a continuously contractive state by ensuring that myosin is not trying to generate a force against actin filaments at all times [16]. However, as there is calcium release into the cytoplasm by virtue of RYR2, the calcium binds troponin C, another protein that's associated with the actin-myosin complex. Due to the proximity of tropomyosin and troponin C, when troponin C binds the calcium, it causes a conformational change in tropomyosin such that it moves relative to the actin filament and reveals binding sites for myosin [35]. Myosin then binds the actin filament and moves to the plus end, causing the shortening of the sarcomere and ultimately the contraction of the heart muscle. Muscle relaxation is driven by the return of calcium into the sarcoplasmic reticulum from the depolarized back into the sarcoplasmic reticulum, by sarcoplasmic reticulum calcium ATPase, or SERCA [19].

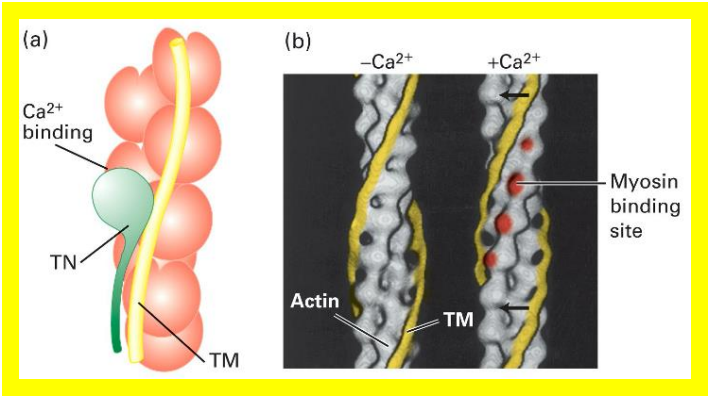

Fig 6: sarcoplasmic reticulum calcium ATPase, or SERCA

3. The Biology of RYR2

One of the aforementioned proteins, RYR2, is among the largest ion channels, with a length exceeding 2MDa. It also one of the critical sources of the release of calcium, is encoded by gene *RYR2*. The RYR2 helps move the calcium that is stored in the sarcoplasmic reticulum to the cytoplasm of the cell in the process of CICR. The main function of the sarcoplasmic reticulum is the storage of calcium ions in muscle cells; this calcium is released when the muscle cell is stimulated, and this release often happens through the RYR2 [29].

The RYR2 channel functions by homo-tetramerizing with other RYR2 receptors on the surface of the sarcoplasmic reticulum, and the additional binding with accessory proteins including calcium, magnesium, protein kinase A, FKBP proteins, calmodulin, calsequestrin-2, junction, treading, and junctophilin-2 – all of which have regulatory roles for the amount intracellular and extracellular calcium that is released through the RyR2 and into the sarcoplasmic reticulum [19][29]. After the tetramerization process and the additional binding with the accessory proteins, the channel is prepared to facilitate the outflow of calcium to excite the muscle cells. In order to have this happen, L-type calcium channels allow the calcium to first bind to the RYR2 that is on the sarcoplasmic reticulum; this initial binding then releases calcium through the RYR2 channel into the cytoplasm. After the calcium is released into cytoplasm, it binds to troponin, which (through tropomysosin) allows myosin to bind to actin, which then contributes contract the cardiac muscle.

RYR2, however, is not characterized by a universally consistent structure. It is the common target of post-translational modifications and especially genetic mutation, and ultimately this then can lead to differences in the amount of calcium that is released into the bloodstream, which can then result in clinical conditions requiring intense treatment [30] . Cytoplasmic area of RYR2 is about 200 angstroms in diameter, connected to the trans membrane domain of about half that size [19] . The corners of the protein that are in touch with the cytoplasmic of the cardiac myocyte are commonly referred to as the ‘clamps,’ and consist of majority alpha helices, which collectively function to maintain connection with the trans membrane domain that is also characterized by longer alpha helical chains[33]. The clamps undergo radical structural changes as they change between the ‘open’ conformation that allows the passage of calcium, and the ‘closed’ conformation that stops the passage; these sites are also especially important in the consideration of modulation through binding with other RYR channels as well as the binding of other smaller ions and regulators.

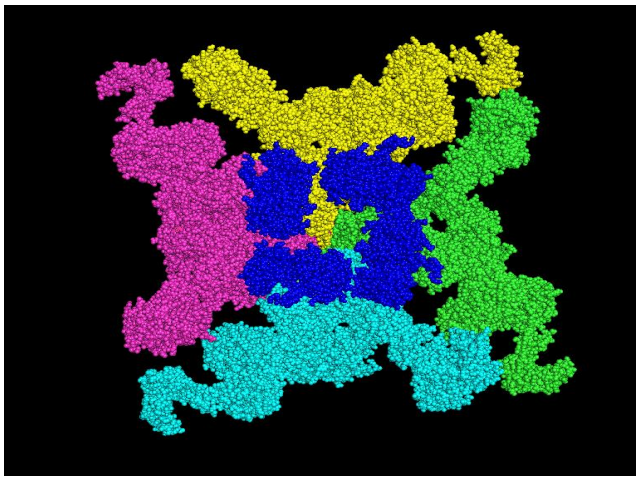

Fig 7: RYR channels closed

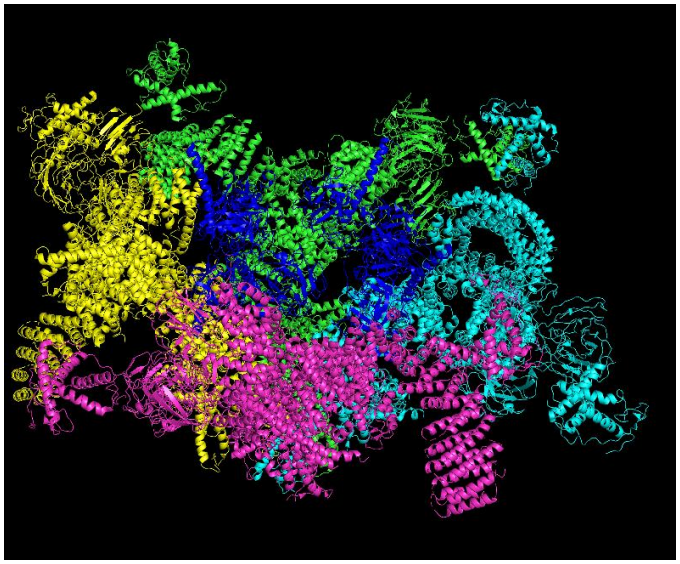

Fig 8: RYR channels closed

4. Mutations in RYR2

Mutations in RYR2 have been associated with many debilitating cardiac illnesses. Mutations associated with issues in cardiac homeostasis in have been shown to be especially devastating manifesting often in illnesses such as catecholaminergic polymorphic ventricular tachycardia (CPVT), arrhythmogenic right ventricular dysplasia type 2 (ARVD2)[1][36] .

While CPVT is more discussed in the context of RYR2, ARVD2 (manifesting in patients as stress-induced ventricular tachycardia) has been connected to RYR2 through the rigorous study of several putative mutants that are associated with the conventional phenotype (Roux-Buisson et al., 2014). These mutations have been associated with a leak of calcium into the T-tubules and a lowered concentration of calcium concentration in the sarcoplasmic reticulum – eventually leading to heart failure as a result of arrhythmogenesis – due to the fact that combinations of as few as two such point mutations have been shown to alter the binding dynamics [22].A similar sort of effect can be seen in CPVT, as putative genetic variation manifest in the form of structural protein changes that eventually change the dynamic of calcium intake and storage. CPVT is well associated with RYR2, with over 50 RYR2 mutations pointing to the manifestation of ventricular arrhythmias among patients [18] . Studies have suggested that it is influenced by binding affinity with regulators have been theorized to have come as a result of specific point mutations within the amino acid sequence, altering protein structure and perhaps the binding affinity associated with regulators that maintain calcium homeostasis[22].

For instance, a point mutation that results in a cysteine at position 4496 within the protein, replacing the wild type arginine, has been known to alter the conformation to allow RYR2 to differentially adopt ‘open’ conformation rather than the ‘closed’ conformation at lower concentrations of calcium but not higher intracellular concentrations of calcium, restricting appropriate concentrations of calcium to enter the cell during the depolarization process, causing arrhythmia [42].

However, the opposite effect has been observed as well, with mutations causing a reduced penetrance among a population subset. Studies suggest that a change from glycine to serine at position 357 has been known to lead to incomplete penetrance of disease and therefore a lower likelihood of diagnosis [21]. The G357S mutation leads to the under-expression of cells that exhibited an overabundance of calcium

in cells, one of the common causes of the arrhythmic phenotype. Upon further investigation, it turned out that the mutation interfered with the N terminal of the protein, the portion that is responsible for stability, eventually causing the reduced expression of viable RYR2 such that the cell was not able to internalize the amount of calcium in the intracellular stores to be harmful to the body [21][2].

5. Mutations in the N Terminal of RYR2

The N terminal is of special importance in RYR2, as there are several mutations that arise for the aforementioned conditions that arise in that region. Due to the homo tetramerizing nature of the protein, all N terminal domains for all four identical chains fall into one region; in addition, due to the fact that the protein binds with three others of its kind any genetic mutations, even those that result in single amino acid substitutions, have profound impacts on the structure and eventually the function of the tetramer. Part of the reason for the enhanced emphasis on the N terminal is due to the fact that it contains a domain peptide that activates the channel. The central helix of the N terminal not only binds to endogenous ions and molecules, but also to other ligands that help modulate the activity of RYR2.

This central helix, when mutated, demonstrates similar characteristics that are often indicative of many of the life-threatening illnesses that come as a result of phenomena, as well as others. Hyper activation of the channel can lead to increased binding of RYR2 to its modulators, while structural locking in the open conformation can lead to the intake of much more calcium than was previously anticipated [18]. More importantly, it has been found that mutations within the N terminal of the tetramer has serious consequences for its ability to bind to its monomer counterparts, compromising its structural integrity and lowering its potential for consistent function – leading to arrhythmogenesis [14] [44] . Interestingly enough, this central helix is well conserved among mammalian species, indicating possible necessity of function has transcended bounds of evolution. The specific function of the central chain can be with led down further from several hundred chains to just 28; between 410 and 438 lies one of the most essential sequences for stability of the N terminal, which in turn allows for the stability of the entire monomer and possibly the tetramer. Structural weakness in channel has the possibility to allow the channels to be locked in an open conformation, causing constitutive calcium signalling [14] [44]. Ultimately, it has been determined that energetics have a lot to do with mutative nature of the N terminal of RYR2; due to the fact that it requires a substantial amount of energy to change from the open conformation to the closed conformation; this suggests that there will be a greater probability for the open conformation [25]. Changes in these energies is caused by a diverse network of hydrogen bonds; while there is more hydrogen bonding interaction in the closed conformation, the energy barrier required to form these hydrogen bonds to eventually stabilize the closed interaction is so great that there is a preference for the open conformation, a preference that eventually manifests itself in a higher probability of finding the receptor in an open conformation – with mutations imminent to confirm this phenotype [17][6] .

Such hydrogen bonding dynamics in the N terminal of RYR2, causing the predominance of open conformation of the tetramer has powerful clinical consequences. Many other mutations described in past studies suggest the gain of function changes that result in more diseased patient outcomes, especially those that increase activity RYR2, increase the frequency of depolarization, or allow for greater intracellular storage of calcium – all of which disrupt the natural flow of calcium and can result in a chronic cardiac condition for the patient.

6. Conclusion and Future Directions

Evidently, per the previous discussion regarding G357S mutation as well as those on the central helix, gain of function mutations may be a large focus of the field but are certainly not the only molecular abnormality that RYR2 undergoes. As such, the field has begun to evolve toward the quantification of calcium inside the heart in order to predict not only the effect of mutated cellular components on the calcium depolarization and muscle contraction cycle on the heart, but additionally to determine short term and longer-term consequences of this concentration on the patient's health. Deep learning algorithms, for instance, have begun to shed light on risk of cardiac problem through the projection of a coronary artery calcium score, eventually using the trends in individualized patient cardiac health to elucidate risk toward a cardiac event [41]. Such quantification has powerful consequences for the diagnosis of conditions relevant to the cardiac arrhythmic concentration of calcium in the heart – CPVT and ARVD2 among them. In addition to deep learning algorithms, there is additionally a push for more chemical approaches to the calcium sensitivity through an understanding of the chemical kinetics that underpin molecular action and molecular effect. Medical imaging is not far behind, with tomography techniques utilizing enhanced CT techniques to determine the amount of calcium present in coronary arteries [43].

Mathematical modelling holds additional promise, especially in light of genetic technologies. Upon identification of mutation in a patient's genotype, math modelling is emerging as a powerful tool to predict concentration of calcium within cardiac cells – which will impact the patient later in life as their condition worsens. Such progression is naturally not completely uniform; as such, research has been done to manipulate math modelling of calcium within the cell to include natural biological waves and oscillations of cytosolic calcium in order to better adapt the patient's prognosis. A notable application of such analysis is the finite element method, a math model that allows solution for a more three dimensional and spatial analysis calcium with in a cell; application of this method on cardiac myocytes and the associated calcium systems with the sarcoplasmic reticulum has the potential to accurately determine the impact of specific mutations on distribution of calcium within the cell [26]. This has far reaching implications for achieving a nuance in diagnosis that can well supplement the clinical standard. As the field begins to embrace alternate cellular mechanisms in addition to the predominant gain of function and associated hyper-action, the further quantitative confirmation of cellular phenomena may serve to shed more light on the molecular actions underpinning debilitating chronic conditions such as those caused by faulty RYR2 action and their role in calcium passage.

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