Conducting research in the Biochemistry and Molecular Biology Laboratory of Dr. Douglas Root, I have contributed to a better understanding of genetic heart diseases and their common symptom, sudden cardiac death. Hypertrophic Cardiomyopathy (HCM) is the most common genetically transferred heart disease, as suggested by epidemiological studies, affecting risk stratification for sudden cardiac death from HCM mutations. These studies have established the relative risk posed by each HCM mutation. Still, only limited research on tropomyosin (an essential molecule in regulating the contraction of heart muscles) has addressed the effect of structural changes in tropomyosin caused by HCM mutations. Although these studies have described the changes in the general structural change of tropomyosin caused by the HCM mutations, they do not explain the detailed changes in molecular structure caused by the HCM mutations. My research aims to fill that void of knowledge by using computational modeling and molecular dynamics (time-dependent simulations) to simulate the effects of HCM mutations on tropomyosin.

This kind of research is urgent because Hypertrophic Cardiomyopathy is the most prominent cause of sudden cardiac death in young adults. Although HCM generally does not exhibit overt symptoms, the expression of the disease is often sudden cardiac death. Such deaths may be traced to the alteration of genes responsible for producing muscle-related proteins, resulting in a change in the heart’s ability to pump blood. Trying to find the effect instigated by these mutations, I have concentrated on tropomyosin, which contains regions where mutations are prominent.

Previous experimental research involving three tropomyosin mutations has supported the common hypothesis that mutations result in structural instability, which in turn cause symptom of HCM. My computer modeling of these mutations are consistent with this idea. However, after I studied other known mutations using the same approach, it appeared that some mutations could actually stabilize the tropomyosin structure. This finding is significant because it may lead to additional research investigating the effects of drugs on the stability of the mutated tropomyosin, thereby contributing to a cure for the disease.

I have therefore conducted two procedures to compare the structural change in tropomyosin induced by HCM: I tested the stability by conducting simulations on both the tropomyosin sections available in current literature. Since no full-length crystallographic atomic models on tropomyosin are currently available in the literature, I created one by combining these structures and modeling the missing parts with its protein sequence, as other researchers have done for other forms of tropomyosin. I reasoned that conducting simulations on the full-length tropomyosin would help eliminate the destabilizing effect all too common around the truncated parts of molecules. I hypothesized as much because tropomyosin sections do not contain the information on the stability of the whole molecule. By way of analogy, for the purpose of clarification, consider a power outage caused by a severed wire. To obtain a full understanding of the effect of the outage, one would have to observe the effect of broken wire and the effect of the outage on people’s households. Similarly, in order to understand the full structural impact of HCM mutations on the tropomyosin, I had to study tropomyosin in the context of both the section and the full molecule.

When I analyzed the location of the point of mutation

![FIGURE 1, 2: Contact map on the crystal structures.](image)

Each point on the chart represents a point of contact chains on tropomyosin sections. In the figure 1 (left), the region of instability around residues 55-78 are stabilized after the introduction of mutation Glu62Gln. In the figure 2, the region around residues 165-178 are destabilized after mutation Glu180Gly is introduced.
eleven mutations that have been documented, I realized there were two locations in tropomyosin where more HCM mutations reside, sort of "hotspots." The first location spans residues 62-95, and the second spans residues 172-192. I chose to investigate selected mutations in each location to determine the structural changes caused by the mutations. By using Maestro (molecular modeling software created by Schrödinger LLC), I analyzed five HCM mutations’ effect on the tropomyosin crystal structures – E62Q, A63V, R70T in the first location; and then E180G and E192K in the second location. I conducted dynamics (time-dependent) simulations on both the mutated and the original copies of two crystal structures, one for each location. I used the deduced crystal structure from chickens (which spans from residue 1-80) for the first location, and the crystal structure from rats (which spans from residue 98-208) for the second. Observing the subtle differences between the wildtype (control) tropomyosin sections and the sections containing HCM mutations, I noted that mutations E62G and A63V cause the molecule to gain contacts between chains of the molecule, a process that causes stability. Mutations R70T, E180G, and E192K cause the molecule to lose the contacts, which causes instability (see Figure 1 and 2 for the stabilizing mutation, E62Q, and the destabilizing mutation, E180G’s results). The correlation between structural changes and HCM suggests that structural change caused by mutations is an important contributor to sudden cardiac death.

I created my own model of the full-length tropomyosin by combining the crystal structures of chicken, rat, and rabbit with the predicted section I created by analyzing the human genetic code for tropomyosin. I have used this full-length model to test mutations for both of the "hotspots." I have, to date, conducted simulations on full-length tropomyosin with one mutation (E62Q); these simulations are also consistent with my previous data. Seeing the results from both the full-length tropomyosin and the tropomyosin sections, I concluded that the stability change of tropomyosin leads to a change in its sensitivity to signals responsible for controlling heart function, which then leads to a stress response in the heart, causing the left side of the heart to enlarge and induce sudden cardiac death. Relative to this process, my simulations have shown that the destabilizing and stabilizing effects of HCM mutations may contribute in significant ways to the ultimate cause of sudden cardiac death.

Notes