weak atrium /myosin heavy chain 6 (wea) Mutation Affects Cardiac Contractility and links to Cardiomyopathy in Zebrafish

Shaline Mahadeo and Dr. Nathalia Holtzman

Department of Biology, Queens College and Macaulay Honors College

Abstract

Cardiomyopathy is a large subset of heart diseases and deformities. The myocardium may enlarge, thicken or become rigid, weakening the heart and its ability to supply the body with oxygenated blood. A host of etiologies lead to cardiomyopathies, and one, in particular, is a mutation of the *weak atrium/myosin heavy chain 6 (wea/myh6)* gene. Homologs of this gene can be found in zebrafish (*Danio rerio*), allowing us to use this organism as a model to study the disorder. Most studies have investigated recessive *wea* mutants, but our lab possesses a unique dominant *wea* mutant. Both variants result in altered atrial-specific phenotypes, disrupting myosin fibers of sarcomeres. The ventricle is also secondarily affected by diminished blood flow and contraction from the atrium. Cardiac chambers may experience edemas, hypertrophy, or dilation. This research project examines the pathogenesis of cardiomyopathy using past literature from both dominant and recessive *wea* variants, while adding insights from our recent work. The similarities of the variants show clinical manifestations of these disorders and their differences illuminate how allelic and external mechanisms dictate morphological phenotypes and disease severity. Together, this information will allow us to expand our understanding of cardiomyopathy and equip us with the knowledge to devise therapeutic approaches for human conditions.

Background

Early stages of cardiogenesis in zebrafish closely mimics human cardiac development and require an interplay of hemodynamic and contractility forces to produce a viable organ. A series of important cell migrations must also occur. It begins with a linear heart tube with an inner endocardium surrounded by an outer myocardial layer of muscle. The transformation of this larval form into the asymmetric adult heart will include a shift to be localized more towards the left and looping and ballooning will then place the atrium to the left of the ventricle. Looping involves the movement of the heart tube to the embryo's right side. The result is the transformation of this initial straight tube into a curved, S-shaped loop with an inner and outer curvature. Ballooning takes place simultaneously and is a swelling or bulging of the tubular walls of the heart.

These series of movements are heavily dependent on physical forces such as blood flow, pressure and rate, which induce region specific cell shape differentiation and patterns of cell division. The pressure and shear forces of the blood flow, determined by the atrium, also has subsequent effects on the morphology of the ventricle. It regulates ventricular cell enlargement, elongation, and proliferation, in specific regions along the curvatures of the heart. Previous studies have implicated the w*eak atrium /myosin heavy chain 6 (wea)* as a key contributor in proper zebrafish cardiogenesis. Recessive and dominant mutations of the gene have been identified, which contribute to cardiomyopathies. A range of phenotypes may result and identifying what factors contribute to which manifestations will potentially direct therapies for cardiomyopathy.

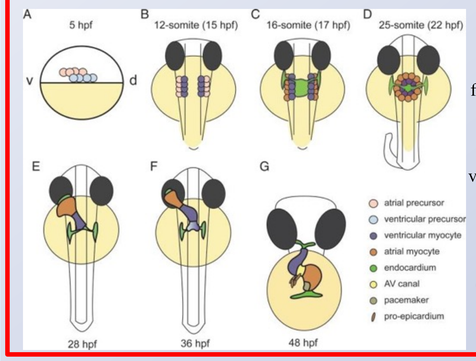

***Figure 1* : Overview of Zebrafish Cardiac Development**

(A-C) Cardiogenic differentiation is initiated in future ventricle cells by expressing cardiac myosin (purple) at 12 hpf. The bilateral heart fuses at the midline, forming a cardiac disc structure with endocardial cells at the hole at the center,, ventricular myocytes at the circumference and atrial myocytes at the periphery (D -E) Cardiac disc transforms into cardiac tube, with endocardium forming the inner lining (F) Differentiation continues at the arterial pole. Cardiac looping displaces the ventricle to the midline and the tube forms an S-shaped loop (G) Pro-epicardial ellipsoid cells cover the myocardium

Methods and Results

Our research analyzes a unique *wea* mutant. It is a similar loss-of-function *atrial myosin heavy chain* mutation, but it is unique because it follows a dominant Mendelian pattern. As a result, we have the advantage of working with three phenotypes rather than two. The first category is the homozygous recessive or wild-type zebrafish/full-contractors (FC). They exhibit healthy, rhythmic contractions of their atrium and ventricle and a strong, pulsating flow of blood circulating throughout their transparent embryos.

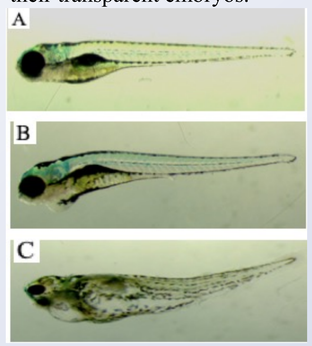

Figure 2. Lateral views of the three different zebrafish phenotypes of the dominant wea mutation.

(A) Normal wildtypes, full-contractors (FC) embryo at 48 hpf

(B) Heterozygotes, partial contractors (PC) embryo at 96 hpf

(C) Homozygous dominant, non-contractors (NC) embryo at 48 hpf

Heterozygotes, or partial-contractors (PC) may present moderate-sized edemas around their chest cavity, or no symptoms at all. These two outcomes generally occur at a 50-50 distribution. Some PC contain an elongated and dilated atrium, but the ventricles appear normal.

Measurements found that PC hearts had an estimated spherical index of 0.69, compared to 0.83 in FC. Measurements of ventricle angle, or the outflow tract angle (OT), which is usually a value of 35° in a wildtype zebrafish, was found to be around 24.6° in PC, illustrating the more linear structure of the heart in mutants and a failure of proper looping and ballooning of the prenatal heart tube.

The third class is the homozygous dominant, non-contractors (NC). These fishes always have massive chest and abdominal edemas. Both the atrium and ventricle produce weak and irregular contractions, and blood flow becomes anemic. This is the result of hypertrophy and insufficient cardiomyocyte elongation in the ventricles. The heart itself will appear more similar to the embryonic tube rather than bulbous chambers when viewed laterally. NC typically do not survive into adulthood.

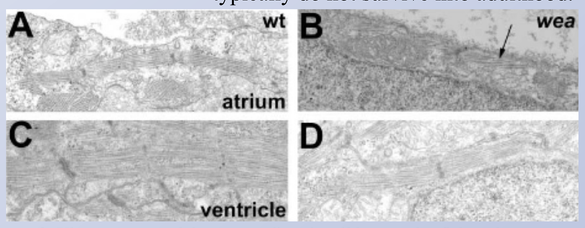

Figure 3. Ultrastructure of the myocardium in wild-type zebrafish and wea mutants.

(A) Wildtype atrium showing striations of tightly organized myofibrils, into sarcomeres.

(B) Atrium of *wea* mutants showing a few, disorganized myofibrils.

(C) Wild type ventricle showing striations of tightly organized myofibrils.

(D) Ventricle of *wea* mutants, showing normal myofibrillar arrays

The origin of the effects of both the recessive and dominant *wea* mutations is traced back to a loss-of-function of the *atrial myosin heavy chain (amhc)* gene, which encodes for myosin heavy chain proteins that comprise atrial sarcomeres. The *wea* locus contains this *amhc* gene, encoding the thick and thin myofibrils of the atrium. The atrium of recessive NC had disorganized myofibrils and sarcomeres in the myocardium. These contractile complexes were sometimes absent altogether in double mutants. The ventricle, however, stains normally and exhibits well-organized sarcomeres, similar to those found in the wildtype.

By compromising the sarcomeric structure of cardiac tissue in the atrium, impaired function is inevitable. Without having the contractile unit to pump the chamber as it ought to, blood flow and blood pressure both decrease in the atrium, and thus, reach the ventricle at diminished force and rate.

Discussion

Our dominant zebrafish *wea* model is especially advantageous to study since many types of cardiomyopathy, including hypertrophic and dilated, have dominant familial inheritance patterns. These PCs can either develop normally into adulthood and display parallel morphology to wildtypes, or they can contain moderately compromised cardiovascular function and visible changes. Understanding what forces determine these outcomes may allow us to pivot dominant human phenotypes into the direction of the wildtype, thereby overcoming the effects of cardiomyopathy.

Some hypotheses have been proposed to explain why some embryos with a dominantly affected allele, will still develop as wildtype. For example, it might be caused by a case of haploinsufficiency of the gene mutation, whereby the one healthy, wildtype copy of the cardiac-regulatory gene is sufficient to circumvent the negative effects of the diseased allele. Another theory might be that in *wea* mutants, ventricular myosin might be up-regulated and may contract faster to compensate for the sub-standard function of the atrium. It is also possible that the *ventricular myosin heavy chain 7* gene, which was previously thought to be solely localized to the ventricle, could migrate and be expressed in the atrium during *wea* mutations, as part of the cell's defense mechanism against atrial dysfunction. As our research with the dominant models of the *wea* mutation continues to progress, these hypotheses can be tested for verification. By unraveling how some cells respond to the mutations of their morphologic regulatory genes, we might be able to use this knowledge to address analogous cardiomyopathy and other cardiac wound healing in humans.

Therapeutic Approaches

Establishing the appropriate hemodynamic forces has shown to be critical in the proper formation of heart chambers. Hence, methods of increasing blood pressure, flow and contractile rate might aid in rescuing *wea* mutants, both PC and NC. Raising the temperature could accomplish this and previous studies have shown promising data. Caffeine and other stimulants such as nicotine can also be explored in terms of how their addition will increase heart rate, blood pressure and metabolism. The *myh6* heterozygotes would be a good sample to perform these experiments on, by observing whether or not these treatments will lead to a greater percentage of PC having better outcomes, in turns of heart physiology and anatomy, as well as overall life expectancy.

Conclusion

As cardiomyopathy gene panels continue to evolve, we are getting a clearer picture about the mechanism and importance of these key regulatory genes that affect form and function of the heart. In the zebrafish model, we see how several landmark genes, including *myh6* regulate chamber formation and ultimately the heart's ability to do work. They have illuminated the importance of contractile force and hemodynamic forces as important players in cardiogenesis. These simple *wea* mutations have big implications and can be analogous to human mutations that produce devastating forms of cardiomyopathy. By investigating the zebrafish models, we can unravel the pathology of cardiomyopathies and open the door for lifesaving new therapies.

References

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