Perspective

Significance of α-Myosin Heavy Chain (MYH6) Variants in Hypoplastic Left Heart Syndrome and Related Cardiovascular Diseases

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Abstract: Hypoplastic left heart syndrome (HLHS) is a severe congenital heart disease (CHD) with complex genetic inheritance. HLHS segregates with other left ventricular outflow tract (LVOT) malformations in families, and can present as either an isolated phenotype or as a feature of a larger genetic disorder. The multifactorial etiology of HLHS makes it difficult to interpret the clinical significance of genetic variants. Specific genes have been implicated in HLHS, including rare, predicted damaging MYH6 variants that are present in >10% of HLHS patients, and which have been shown to be associated with decreased transplant-free survival in our previous studies. MYH6 (α-myosin heavy chain, α-MHC) variants have been reported in HLHS and numerous other CHDs, including LVOT malformations, and may provide a genetic link to these disorders. In this paper, we outline the MYH6 variants that have been identified, discuss how bioinformatic and functional studies can inform clinical decision making, and highlight the importance of genetic testing in HLHS.

Keywords: hypoplastic left heart syndrome; cardiac myosin heavy chain; congenital heart disease; rare variant analysis

1. Background

Hypoplastic left heart syndrome (HLHS) is a complex form of congenital heart disease (CHD) characterized by hypoplasia of the left ventricle and proximal aorta, as well as stenosis or atresia of the mitral and/or aortic valves [1]. Although significant evidence exists for a genetic basis of HLHS [2,3], its inheritance is multifactorial, complicating the identification of specific genetic risk factors. HLHS occurs in the context of larger chromosomal abnormalities (e.g., Turner [4] and Jacobson syndromes [5]), but also exists as an isolated disorder [6,7]. Additionally, there is an increased incidence of bicuspid aortic valve (BAV), atrial septal defect (ASD), and other left ventricular outflow tract (LVOT) malformations in family members of HLHS patients. Genes implicated in non-syndromic HLHS include MYH6 [8,9], NOTCH1 [10], NKX2.5 [11], ERBB4 [12], HAND1 [13], and GJA1 [14].
We previously identified 19 distinct, rare, predicted damaging MYH6 variants in a cohort of 190 unrelated HLHS subjects, comprising >10% of the cohort [8]. These findings are consistent with previous studies of mutations in the zebrafish MYH6 homologue, amhc/myh6, wherein loss-of-function mutations, along with amhc morpholino knockdown, disrupted atrial sarcomere assembly, impaired atrial contractility, and resulted in atrial dilation in zebrafish embryos. Mutant embryos also exhibited ventricular wall thickening and a narrowed ventricular lumen, mimicking the HLHS phenotype [15]. The additional characterization of knockdown embryos revealed that abnormal ventricular morphology was not due to differences in cardiomyocyte number, but rather due to differences in the size and shape of ventricular cardiomyocytes in myh6 mutants, compared to wild-type [16]. Similarly, developing myh6−/− Xenopus tropicalis hearts lacked cardiac contractility, which was accompanied by atrial and ventricular dilation, and impaired outflow tract development [17]. Although murine models are widely used to study CHD, their cardiac chamber-specific expression of MHC is opposite that of humans, making them unsuitable for modeling MYH6-associated disease.

MYH6 encodes for the alpha isoform of the cardiac myosin heavy chain (α-MHC), which is expressed throughout the myocardium during early cardiac development. As development proceeds, MYH6 expression decreases in the ventricles and is replaced with MYH7 (β-MHC) throughout gestation; α-MHC is the dominant atrial isoform postnatally [18–20]. Genetic variants in both MYH6 and MYH7 have been linked to numerous human cardiac pathologies, including hereditary cardiomyopathies, arrhythmias, as well as CHD. While MYH7 variants have been characterized more extensively, the specific mechanisms underlying MYH6 variants are less understood. In this paper, we outline the MYH6 variants that have been reported in HLHS and other CHDs, discuss the benefits and limitations of biostatistical methods for interpreting variants, and emphasize the importance of mechanistic studies designed to improve personalized treatment strategies.

2. Genetic Studies

2.1. Known MYH6 Variant Disease Associations

A possible pathogenic role for MYH6 was first reported more than 30 years ago in a family with hypertrophic cardiomyopathy (HCM). A disease locus, “FHC-1”, was initially identified in all affected but no unaffected members of this family [21]. Investigators later determined that the pathogenicity of the FHC-1 locus was due to a hybrid MYH6/MYH7 gene, in which intron 26 of MYH6 was joined to intron 27 of MYH7 [22]. In the decades since, improvements in next-generation sequencing technology and awareness of the significance of MYH6 in CHD has led to discovery of additional disease-associated MYH6 variants through both family-based and CHD cohort studies. Many groups have reported MYH6 variants in association with septal defects [23–27] (most commonly ASD) [28–31], as well as in various types of arrhythmias and sudden cardiac death [32–41]. MYH6 variants are also associated with all types of cardiomyopathy, including HCM [42–46], dilated cardiomyopathy (DCM) [41,47–52], peripartum cardiomyopathy (PPCM) [53], arrhythmogenic right ventricular cardiomyopathy (ARVC) [54], and left ventricular non-compaction (LVNC) [55,56]. MYH6 variants have also been identified in patients with Shone complex [27], mitral valve prolapse (MVP) [57], coarctation of the aorta (CoA) [58], and, of relevance to this review, HLHS [23,25,26,59]. Remarkably, HLHS and other LVOT malformations are associated with 49% of all reported MYH6 variants. A list of published MYH6 coding sequence variants and associated CHDs is shown in Table 1.
Table 1. Published MYH6 coding sequence variants. Minor allele frequencies were obtained from the Genome Aggregation Database (gnomAD) Genomes dataset v3.1.2 and the Allele Frequency Aggregator (ALFA) dataset (release version 20201027095038). Computational methods used to predict deleterious nature of variants include scaled Combined Annotation Dependent Depletion (CADD) score (GRCh37, v1.6) [60], SIFT [61], and PolyPhen2 [62]. Not all computational methods were able to calculate potential pathogenicity scores for truncating variants or deletions. Abbreviations: HLHS, hypoplastic left heart syndrome; ASD, atrial septal defect; CHD, unspecified congenital heart disease; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; TA, tricuspid atresia; LVH, left ventricular hypertrophy; TGA, transposition of the great arteries; PFO, patent foramen ovale; MVP, mitral valve prolapse; PPCM, peripartum cardiomyopathy; SSS, sick sinus syndrome; CoA, coarctation of the aorta; WPW, Wolff–Parkinson–White syndrome; AVSD, atrioventricular septal defect; ARVC, arrhythmogenic right ventricular cardiomyopathy; AF, atrial fibrillation; LVNC, left ventricular noncompaction; SDK, septal dyskinesia; SAR, subaortic ridge; AS, aortic stenosis; DVC, dilated inferior vena cava.

| Variant | Associated Phenotype | Scaled CADD Score | SIFT Prediction | PolyPhen2 Prediction | GnomAD Genomes Allele Frequency | ALFA Allele Frequency |
|---------|----------------------|------------------|----------------|----------------------|---------------------------------|----------------------|
| D3N     | HLHS [63]            | 25.6             | damaging       | probably damaging    | 9.20 × 10^{-5}                 | 4.00 × 10^{-5}       |
| R17H    | ASD [30]             | 26.3             | damaging       | probably damaging    | 6.57 × 10^{-6}                 | not reported         |
| P40R    | CHD [23]             | 20.6             | damaging       | probably damaging    | not reported                    | not reported         |
| E98K    | Shone complex [27], HLHS [63], CHD [26] | 27.4 | damaging | probably damaging | 3.15 × 10^{-4} | 6.12 × 10^{-4} |
| R108C   | HLHS [63]            | 29               | damaging       | probably damaging    | not reported                    | not reported         |
| Y115N   | HLHS [8]             | 28.6             | damaging       | probably damaging    | not reported                    | not reported         |
| S118L   | HLHS [63]            | 27.4             | damaging       | possibly damaging    | 6.60 × 10^{-6}                 | not reported         |
| S180Y   | DCM [52]             | 26.4             | damaging       | probably damaging    | not reported                    | not reported         |
| R204H   | HCM [43]             | 23.7             | tolerated      | probably damaging    | 4.60 × 10^{-5}                 | 2.20 × 10^{-5}       |
| D208N   | AVSD [24]            | 22.3             | tolerated      | benign               | 4.15 × 10^{-3}                 | 6.38 × 10^{-3}       |
| A230P   | TA, LVH [26]         | 25.4             | damaging       | probably damaging    | not reported                    | not reported         |
| H252Q   | TGA, PFO [26]        | 22.4             | damaging       | probably damaging    | 2.63 × 10^{-5}                 | 6.00 × 10^{-5}       |
| I275N   | DCM [48,49]          | 17.4             | damaging       | probably damaging    | 1.84 × 10^{-4}                 | 3.63 × 10^{-4}       |
| Q277H   | HLHS [8,63], CHD [23] | 20.9 | damaging | benign | 2.76 × 10^{-4} | 4.04 × 10^{-4} |
| E329stop| ASD [29]             | 43               | damaging       | N/A                  | not reported                    | not reported         |
| D383N   | HLHS [8]             | 25.5             | damaging       | probably damaging    | not reported                    | not reported         |
| S385L   | HLHS [8]             | 23.1             | tolerated      | benign               | 9.87 × 10^{-5}                 | not reported         |
| L388F   | Shone complex [27]   | 24.7             | damaging       | probably damaging    | not reported                    | not reported         |
| G415R   | MVP [57]             | 26.9             | damaging       | probably damaging    | 6.57 × 10^{-5}                 | 7.00 × 10^{-5}       |
| M436V   | HLHS [8]             | 24.6             | tolerated      | probably damaging    | not reported                    | not reported         |
| R443P   | HLHS [8]             | 27.2             | damaging       | probably damaging    | not reported                    | 3.00 × 10^{-5}       |
| E501stop| TA [26]              | 39               | damaging       | N/A                  | not reported                    | not reported         |
| I512T   | Shone complex [26]   | 23.2             | damaging       | N/A                  | not reported                    | not reported         |
| E526K   | ASD [64]             | 27.5             | damaging       | possibly damaging    | 6.57 × 10^{-6}                 | not reported         |
| C539R   | ASD [30]             | 26.3             | damaging       | possibly damaging    | not reported                    | not reported         |
| K543R   | ASD [30]             | 25.4             | damaging       | possibly damaging    | 5.93 × 10^{-5}                 | 1.20 × 10^{-4}       |
| R568C   | PPCM [53], DCM [48]  | 25.7             | damaging       | probably damaging    | 3.29 × 10^{-5}                 | 9.00 × 10^{-5}       |
Table 1. Cont.

| Variant  | Associated Phenotype                  | Scaled CADD Score | SIFT Prediction   | PolyPhen2 Prediction | GnomAD Genomes Allele Frequency | ALFA Allele Frequency |
|----------|---------------------------------------|-------------------|-------------------|----------------------|---------------------------------|-----------------------|
| G585S    | Shone complex [27]                    | 24.4              | damaging          | possibly damaging    | 1.31 × 10^{-4}                 | 1.91 × 10^{-4}        |
| D588A    | HLHS [8,9,63]                         | 22.6              | tolerated         | benign               | 1.40 × 10^{-3}                 | 2.51 × 10^{-3}        |
| V606I    | HLHS [63]                             | 23.1              | damaging          | benign               | 6.57 × 10^{-5}                 | not reported          |
| D629N    | HCM [44]                              | 22.5              | tolerated         | possibly damaging    | 5.91 × 10^{-5}                 | 7.00 × 10^{-5}        |
| F646L    | HLHS [63]                             | 24.4              | damaging          | possibly damaging    | not reported                    | not reported          |
| R654W    | Arrhythmia [33]                       | 31                | damaging          | probably damaging    | 1.97 × 10^{-5}                 | 4.00 × 10^{-5}        |
| N678S    | ASD, TA [25]                          | 25.1              | damaging          | probably damaging    | not reported                    | 7.00 × 10^{-5}        |
| V700M    | PFO [26,65]                           | 26.8              | damaging          | probably damaging    | 6.57 × 10^{-6}                 | not reported          |
| I704N    | HLHS [9]                              | 26.7              | damaging          | probably damaging    | not reported                    | not reported          |
| R721W    | SSS [35], CoA [58]                    | 26                | damaging          | probably damaging    | 1.91 × 10^{-5}                 | not reported          |
| R795Q    | HCM [42]                              | 25.9              | damaging          | probably damaging    | not reported                    | 1.00 × 10^{-4}        |
| R795W    | HLHS [8]                              | 26                | damaging          | probably damaging    | 1.38 × 10^{-4}                 | 2.54 × 10^{-4}        |
| I806T    | DCM [41]                              | 22.6              | tolerated         | benign               | not reported                    | not reported          |
| R809C    | HCM [43]                              | 26                | damaging          | probably damaging    | 1.31 × 10^{-5}                 | 8.00 × 10^{-5}        |
| I820N    | ASD [31]                              | 26.3              | damaging          | not reported         | not reported                    | not reported          |
| P830L    | DCM [47,50]                           | 26.3              | damaging          | probably damaging    | not reported                    | not reported          |
| K849del  | HLHS [8]                              | N/A               | N/A               | N/A                  | not reported                    | not reported          |
| T864M    | HLHS [63]                             | 20.7              | tolerated         | benign               | 7.89 × 10^{-5}                 | 1.40 × 10^{-4}        |
| E885K    | WPW [32]                              | 29.3              | damaging          | probably damaging    | not reported                    | not reported          |
| A895V    | CHD [26]                              | 32                | damaging          | possibly damaging    | 6.57 × 10^{-6}                 | not reported          |
| E933del  | SSS [39]                              | N/A               | N/A               | N/A                  | not reported                    | not reported          |
| A936V    | HLHS [8], AVSD [24]                   | 25.9              | tolerated         | possibly damaging    | 6.24 × 10^{-4}                 | 2.16 × 10^{-4}        |
| E948K    | MVP [57], HLHS [63]                   | 27.4              | damaging          | probably damaging    | 5.91 × 10^{-5}                 | 3.00 × 10^{-5}        |
| C949stop | ARVC [54]                             | 36                | damaging          | N/A                  | not reported                    | not reported          |
| E951stop | ASD [29]                              | 38                | damaging          | N/A                  | not reported                    | not reported          |
| A964S    | HLHS [8]                              | 26.3              | tolerated         | benign               | 1.37 × 10^{-3}                 | 1.76 × 10^{-4}        |
| A1004S   | ASD [30], DCM [47,48,50]              | 23.6              | tolerated         | benign               | 1.10 × 10^{-3}                 | 1.03 × 10^{-3}        |
| R1047C   | DCM [51]                              | 29.7              | damaging          | probably damaging    | 7.23 × 10^{-5}                 | 2.00 × 10^{-5}        |
| R1052stop| MVP [57], HLHS [63]                   | 38                | damaging          | N/A                  | 1.97 × 10^{-5}                 | 3.00 × 10^{-5}        |
| Q1065H   | HCM [47]                              | 23.5              | damaging          | probably damaging    | 1.51 × 10^{-4}                 | 1.10 × 10^{-4}        |
| I1068T   | Shone complex [27]                    | 21.9              | tolerated         | benign               | not reported                    | not reported          |
| R1116S   | ASD [26]                              | 24.6              | damaging          | probably damaging    | 7.90 × 10^{-5}                 | 7.00 × 10^{-5}        |
| R1116C   | HCM [66]                              | 26                | damaging          | probably damaging    | not reported                    | not reported          |
| R1116H   | SSS [67]                              | 25                | damaging          | probably damaging    | 4.60 × 10^{-5}                 | 7.00 × 10^{-5}        |
| R1151Q   | HLHS [8]                              | 28.9              | tolerated         | probably damaging    | 1.32 × 10^{-5}                 | not reported          |
| R1177W   | DCM [48]                              | 25.4              | damaging          | probably damaging    | 2.64 × 10^{-5}                 | 4.00 × 10^{-5}        |
| T1190I   | HLHS [63]                             | 25.4              | damaging          | probably damaging    | 3.95 × 10^{-5}                 | not reported          |
Table 1. Cont.

| Variant | Associated Phenotype | Scaled CADD Score | SIFT Prediction  | PolyPhen2 Prediction  | GnomAD Genomes Allele Frequency | ALFA Allele Frequency |
|---------|----------------------|-------------------|------------------|-----------------------|-------------------------------|----------------------|
| E1207K  | HLHS [9]             | 27.3              | damaging         | probably damaging     | 5.93 × 10⁻⁵                   | 5.00 × 10⁻⁵          |
| R1252Q  | SSS [67]             | 24.9              | damaging         | not reported          | not reported                  | not reported         |
| T1253M  | DCM [51]             | 25.3              | tolerated        | probably damaging     | 1.31 × 10⁻⁵                   | not reported         |
| R1279stop| ASD [28]             | 38                | damaging         | N/A                   | not reported                  | not reported         |
| R1291P  | HLHS [63]            | 31                | damaging         | probably damaging     | not reported                  | not reported         |
| A1298V  | HLHS [8]             | 25                | tolerated        | possibly damaging     | 1.12 × 10⁻⁴                   | 2.00 × 10⁻⁴          |
| K1307M  | AF [34]              | 28.8              | damaging         | probably damaging     | not reported                  | not reported         |
| D1316E  | SCD [37]             | 19.7              | tolerated        | possibly damaging     | 1.31 × 10⁻⁵                   | 3.00 × 10⁻⁵          |
| E1323V  | AF [34]              | 31                | damaging         | probably damaging     | not reported                  | not reported         |
| A1327V  | Shone complex [27]   | 28.1              | damage           | probably damaging     | 6.21 × 10⁻⁴                   | 2.00 × 10⁻⁴          |
| S1337L  | LVNC [55]            | 28.9              | damaging         | probably damaging     | 1.98 × 10⁻⁵                   | 4.00 × 10⁻⁵          |
| A1366D  | SDK, SAR, PFO, AS [26]| 28.9              | damaging         | probably damaging     | not reported                  | not reported         |
| T1379M  | HLHS [8,9], MVP [57], CHD [26]| 27.3 | damaging         | probably damaging     | 3.22 × 10⁻⁴                   | 5.87 × 10⁻⁴          |
| R1398Q  | HLHS [63], CHD [23]  | 23.6              | damaging         | benign                | 4.14 × 10⁻⁴                   | 5.26 × 10⁻⁴          |
| A1440P  | DCM [48]             | 26.4              | tolerated        | possibly damaging     | not reported                  | not reported         |
| A1443D  | ASD [26,65], HLHS [8,63]| 26.3 | damaging         | not reported          | 1.71 × 10⁻⁴                   | 1.30 × 10⁻⁴          |
| E1457K  | DCM [47,50]          | 28.1              | damaging         | probably damaging     | 1.31 × 10⁻⁵                   | not reported         |
| R1502Q  | DCM [48,49]          | 30                | damaging         | probably damaging     | 2.17 × 10⁻⁴                   | 2.61 × 10⁻⁴          |
| E1503V  | HLHS [8]             | 33                | damaging         | probably damaging     | not reported                  | not reported         |
| E1584K  | HLHS [8]             | 28.7              | damaging         | probably damaging     | 2.63 × 10⁻⁵                   | not reported         |
| R1608C  | CHD [23]             | 26                | damaging         | probably damaging     | 4.60 × 10⁻⁵                   | 8.00 × 10⁻⁵          |
| R1610C  | Shone complex [27]   | 28.7              | damaging         | probably damaging     | 7.89 × 10⁻⁵                   | 6.00 × 10⁻⁵          |
| A1674T  | CHD [41]             | 24.5              | tolerated        | benign                | 4.60 × 10⁻⁵                   | 3.00 × 10⁻⁵          |
| E1713K  | HLHS [63]            | 30                | damaging         | probably damaging     | 5.26 × 10⁻⁵                   | not reported         |
| E1754stop| HLHS [8]             | 45                | damaging         | N/A                   | 1.97 × 10⁻⁵                   | not reported         |
| E1827D  | HLHS [63]            | 25.1              | damaging         | probably damaging     | not reported                  | not reported         |
| K1840R  | HLHS [8] HCM [43]    | 25.1              | tolerated        | probably damaging     | 1.12 × 10⁻⁴                   | 2.50 × 10⁻⁴          |
| D1859N  | HLHS [63]            | 29.8              | damaging         | probably damaging     | 1.94 × 10⁻⁵                   | not reported         |
| R1865Q  | DIVC, ASD, VSD [26]  | 29.8              | damaging         | probably damaging     | 4.06 × 10⁻⁵                   | 3.00 × 10⁻⁵          |
| A1891T  | Shone complex [27]   | 26.8              | damaging         | probably damaging     | not reported                  | not reported         |
| R1899C  | DCM [48]             | 32                | damaging         | probably damaging     | 1.31 × 10⁻⁵                   | not reported         |
| R1899H  | Shone complex [27]   | 31                | damaging         | probably damaging     | 3.94 × 10⁻⁵                   | 3.00 × 10⁻⁵          |
| R1911P  | HLHS [63]            | 29.6              | damaging         | probably damaging     | not reported                  | not reported         |
| K1932stop| Shone complex [27]   | 51                | damaging         | N/A                   | 1.97 × 10⁻⁵                   | not reported         |
2.2. Clinical Interpretation of Genetic Studies

These genetic studies have been highly informative, but limitations remain in connecting the knowledge of MYH6 variants to clinical phenotypes. In cohort studies, the designation of a variant as disease-causing relies on a combination of allele frequency and bioinformatic tools of pathogenicity, which are subject to change as new information is learned. There is not a single consensus on what constitutes a “rare” variant, or what is considered damaging when using a continuous variant scoring system, such as Combined Annotation Dependent Depletion (CADD). It is even more challenging to interpret the significance of variants when there are conflicting assessments between computational predictions and variant frequency, or between multiple predictive methods, for example, the MYH6 variants Q277H, M436V, I512T, V606I, D629N, R860H, A936V, R1151Q, A1298V, D1316E, and R1398Q (Table 1), which have predicted opposite effects when compared using the popular tools SIFT and Polyphen2.

Family-based studies remain a useful way of identifying pathogenic variants, as the segregation of a gene variant within multiple affected family members provides strong evidence that a variant is disease-causing. Familial studies are also directly informative for clinical practice when determining whether family members of an affected individual should be screened for the variant, and if carriers should be surveilled for future disease development. These considerations are particularly important in HLHS due to its high heritability and segregation with other LVOT malformations in family members [66,67], many of which are also associated with MYH6 variants.

2.3. Impact of MYH6 Variants on Outcomes in HLHS

Our group has examined outcomes of patients with HLHS stratified by presence of an MYH6 variant. Specifically, we compared a composite endpoint of cardiac arrest, need for mechanical circulatory support, and heart transplant or death between 12 HLHS patients with MYH6 and 24 HLHS patients without MYH6 variants. In this cohort, each MYH6 variant carrier was matched to two controls based on anatomical subtype (i.e., aortic and mitral valve anatomy), stage I surgical shunt type, age/era, and sex when possible. Patients with chromosomal abnormalities and those carrying MYH7 variants were excluded from this analysis. The difference in reaching the composite endpoint at 15 years between MYH6 variant and control groups did not reach statistical significance in this small study (Figure 1). However, there is certainly a trend towards improved short-term event-free survival in the control group. Control group outcomes appear better than previously reported transplant-free survival of HLHS patients during follow-up of the single ventricle reconstruction (SVR) randomized trial cohort, which examined differences in transplant-free survival and interventions based on stage I surgical shunt type [68]. These findings warrant further investigation with a larger sample size and emphasize the importance of genetic testing for all HLHS patients to identify variants that may impact survival even more so than surgical shunt type.
transplant-free survival and interventions based on stage I surgical shunt type [68]. These findings warrant further investigation with a larger sample size and emphasize the importance of genetic testing for all HLHS patients to identify variants that may impact survival even more so than surgical shunt type.

Figure 1. Event-free survival analysis comparing 36 HLHS patients. A total of 12 patients had rare, predicting damaging MYH6 variants. p-value = 0.074, log-rank test.

3. Mechanisms of MYH6 Variant Pathology

3.1. Importance of Mechanistic Studies

Understanding the specific mechanism of MYH6 variant pathogenicity would be especially relevant to clinical decision making, considering the availability of the drugs omecamtiv mecarbil and mavacamten, which act specifically on the cardiac myosin heavy chains (MHC) to improve systolic and diastolic function, respectively. In phase III clinical trials, both drugs showed efficacy in the treatment of heart failure in adults [69–72], irrespective of genetic background; omecamtiv mecarbil was FDA-approved for use earlier this year, and FDA approval of mavacamten is pending. In HLHS patients with a known pathogenic MYH6 variant, the cardiac specificity of omecamtiv mecarbil and mavacamten may offer a way to prevent disease progression. This treatment may be particularly important in variant carriers, given our previous report that HLHS patients with MYH6 variants have decreased cardiac transplant-free survival compared to HLHS patients without MYH6 variants [8]. However, choosing to use a cardiac MHC-specific activator vs. inhibitor requires the understanding of whether a specific variant will cause systolic or diastolic dysfunction. This highlights the importance of mechanistic studies designed to understand phenotypes at the cellular and tissue levels. Relative to the large body of literature assessing MYH7 variants, few studies have sought to understand MYH6 variant pathology at the molecular level; the findings from these studies are summarized in Table 2.

Table 2. Functional studies evaluating the role of MYH6 variants in cardiac disease.

| Model System            | MYH6 Variant | Disease Association | Cellular Phenotype                         | Reference |
|-------------------------|--------------|---------------------|--------------------------------------------|-----------|
| Purified recombinant    | I820N        | ASD                 | • ↓ binding affinity of α-MHC to RLC       | [31]      |
| protein                 |              |                     |                                            |           |
| HeLa cells              | E933del      | SSS                 | • ↓ binding affinity of α-MHC to MyBP-C    | [39]      |
| Model System | MYH6 Variant | Disease Association | Cellular Phenotype | Reference |
|--------------|--------------|---------------------|--------------------|-----------|
| **Myofibrils differentiated from C2C12 myoblasts** | | | | |
| | A230P | TA, ASD, LVH | • ↓ myofibrillar organization | [26] |
| | A1366D | AS, SDK, SAR, PFO | • ↓ myofibrillar organization | [26] |
| | H252Q | TGA, PFO | • ↑ myofibril striations | [26] |
| | V700M | PFO | • No effect on myofibrillar organization | [26] |
| | E526K | ASD | • ↓ myofibrillar organization | | No impact on actin-activated ATPase activity | [64] |
| | R1822_E1823dup | ASD | • ↓ myofibrillar organization | | No impact on cell viability | | ↑ apoptosis | [73] |
| | R721W | SSS, CoA | • ↓ myofibril striations | | MHC aggregation | | | [39] |
| | P830L | DCM | • No effect on peak contraction, shortening velocity, Ca homeostasis, or relaxation time | [74] |
| | E933del | SSS | • ↓ propagation velocity | | • ↓ myofibril striations | | • MHC aggregation | [39] |
| | A1004S | ASD, DCM | • ↓ peak contraction (with and without isoproterenol stimulation) | | • ↓ shortening velocity | | No change in Ca homeostasis or relaxation time | [74] |
| **Induced pluripotent stem-cell-derived cardiomyocytes (iPSC-CMs)** | | | | |
| | R443P | HLHS | • ↓ cardiomyogenic differentiation | | • Disorganized sarcomeres | | • ↓ shortening and relaxation rates | | • ↓ extent of shortening, % shortening | | • ↓ amplitude of Ca transient | | • No effect on action potential | | • ↑ expression of sarcomere genes, including MYH7 (β-MHC) | [8,75] |
| | R443P | HLHS | • Atrial sarcomere disarray | [8] |
| | N598fs | ACM | • LV and septal fibrosis | [54] |
| | D629N | HCM | • RV and septal myocyte disarray | [44] |
| | A822T | SCD | • LV and conduction system fibrosis | [36] |
| | K849del | HLHS | • Atrial sarcomere disarray (no effect on ventricular sarcomere organization) | [8,75] |
| | E1503V | HLHS | • Atrial sarcomere disarray (no effect on ventricular sarcomere organization) | [75] |
| | S385L & M436V | HLHS | • No effect on atrial or ventricular sarcomeres | [75] |
Table 2. Cont.

| Model System | MYH6 Variant | Disease Association | Cellular Phenotype | Reference |
|--------------|--------------|---------------------|--------------------|-----------|
| Zebrafish    | E933del      | SSS                 | • ↓ heart rate of MYH6 knockout was rescued by wild-type MYH6, but not variant | [39] |
|              | R1252Q       | SSS                 | • ↓ heart rate, stroke volume, cardiac output, fractional area change of MYH6 knockout were rescued by wild-type MYH6, but not variant | [67] |

3.2. In Vitro Mechanistic Studies

Most of the mechanistic studies of MYH6 variants have utilized in vitro methods. The first variant to be functionally assessed was MYH6-I820N; this ASD-associated variant is located within the regulatory light chain (RLC) binding region of α-MHC and was found in cellular studies to decrease the binding affinity of α-MHC for RLC [31]. However, RLC binding is thought to modulate MHC activation [76] and it is unclear how a disruption in this process could lead to an ASD. Similarly, the SSS-associated MYH6-E933del variant enhanced binding to myosin-binding protein C (MyBP-C), an effect consistent with the location of E933del within the MyBP-C binding region of the α-MHC protein [39]. HL-1 mouse atrial cardiomyocytes transfected with human MYH6-E933del also exhibited a slower electric propagation velocity compared to human MYH6-WT [39], and neonatal rat ventricular cardiomyocytes (NRVCs) transfected with either the E933del or the R721W [35] variant exhibited disrupted sarcomere structure and the perinuclear aggregation of α-MHC [39]. Together, these findings led the authors to suggest that structural changes within the atrial cardiomyocytes surrounding the sinus node leads to node dysfunction and conduction defects. However, given the role of MyBP-C in modulating contractile strength [77], it is unclear how changes in the α-MHC/MyBP-C interaction are linked to the identified conduction deficits.

Many other MYH6 variants have shown similar changes in sarcomere structure. Cultured cardiomyocytes expressing the A230P [26], A1366D [26], E526K [64], R1822_E1823dup [73], and HLHS-associated R443P [8] variants exhibited decreased sarcomere organization in variant-carrying cells, while H252Q actually increased myofibril striations [26]. Interestingly, the MYH6-V700M variant did not appear to impact sarcomere organization, despite its rare frequency (<0.001%) and being predicted as “likely damaging” by CADD, SIFT, and PolyPhen2. Our lab also assessed sarcomere organization in cardiac tissue from HLHS patients and found that atrial sarcomeres were disrupted with the R443P, K849del, and E1503V variants, while the ventricular sarcomere structure remained intact [75], consistent with α-MHC being the predominant atrial MHC isoform postnatally. Other groups have also evaluated cardiac tissue from MYH6 variant carriers and reported fibrosis in the conduction system [36], ventricular walls [36,44,54], and ventricular septum [44,54]. Given the predominance of β-MHC in the postnatal ventricles, it is possible that ventricular fibrosis is, at the cellular level, a downstream response to atrial cardiomyocyte dysfunction caused by MYH6 variants in the patients studied.

Some of the most informative in vitro studies are those that examine contractility at the cellular level. NRVCs expressing human MYH6-A1004S shortened at a slower rate, and consequently shortened less overall, when compared to NRVCs expressing human MYH6-WT. In the same study, NRVCs expressing human MYH6-P830L showed no difference in shortening rate, compared to MYH6-WT [74]. This finding is somewhat unexpected, given that A1004S is located on the MHC backbone and is predicted by both SIFT and PolyPhen2 to be non-damaging (Table 1). The MYH6-A1004S variant is also found at a frequency of 1.1% in the general population, which is more common than CHD.
Meanwhile, MYH6-P830L is a novel variant and located near the RLC binding region and thus one would predict greater changes in function with P830L than A1004S. Our lab also found that the MYH6-R443P variant decreased the shortening rate, relaxation rate, extent of shortening, percent shortening, and calcium transient amplitude at the single CM level in patient-specific induced pluripotent stem-cell-derived cardiomyocytes (iPSC-CMs), without affecting action potentials. These MYH6-R443P iPSC-CMs also demonstrated sarcomere disorganization and the upregulation of MYH7, recapitulating the phenotype found in atrial tissue from an HLHS patient carrying the R443P variant [8,75].

3.3. In Vivo Mechanistic Studies

To date, zebrafish embryos are the only animal model that has been used to study human MYH6 variants. Specifically, researchers evaluated the ability of the human MYH6-E933del and MYH6-R1252Q variants, which are associated with cardiac conduction disease, to rescue cardiac impairments resulting from myh6 knockdown. In both sets of experiments, the authors reported bradycardia in knockdown embryos at 48 hpf −137.7 ± 2.2 bpm in myh6−/− vs. 150.2 ± 1.6 in uninjected [39], and 144 ± 16 bpm in myh6−/− vs. 153 ± 13 in uninjected [67]. While human MYH6-WT and MYH6-R1252Q increased heart rate in knockdowns, human MYH6-E933del failed to rescue this phenotype.

3.4. Structural Considerations

The structure of human α-MHC has not been solved, thus most hypotheses regarding the effect of MYH6 variants on the α-MHC structure are based on comparison to the solved structures of β-MHC. Mutational clustering analysis using population-level data found that pathologic variants in MYH7 cluster in certain regions [78], which has been used to inform ACMG/AMP variant classification framework [79]. At present, no such “mutational hotspots” have been identified in MYH6 (Figure 2); however, similar patterns could emerge as new pathological variants are discovered. Investigating structure-function relationships in MYH7 has been successful in elucidating a mechanism for HCM that results from variants in a surface region of β-MHC referred to as the “myosin mesa” [80,81]. However, the interpretation of such studies may be complicated by the finding that the same MYH7 variant can cause clinically opposite phenotypes (i.e., both HCM and DCM), depending on the person [82].

Some research groups have begun employing advanced in silico methods to model the effect of specific MYH6 variants. Molecular dynamics simulations predicted that the MYH6 variants E1207K and T1379M alter the helicity and flexibility of the tail domain, which would likely impact the rigidity and movement of the thick filament as a whole [9]. Similarly, simulations found that MYH6-R1822_E1823dup likely increases the strength of the dimerized α-MHC tail domain, decreasing its flexibility [73]. However, this information does not explain either the clinical or cellular phenotypes associated with these variants.
Variants were considered rare if allele frequency was <1 × 10^{-3} in both the Genome Aggregation Database (gnomAD) Genomes dataset v3.1.2 and the Allele Frequency Aggregator (ALFA) dataset (release version 20201027095038). Single nucleotide variants were considered predicted damaging if the scaled Combined Annotation Dependent Depletion (CADD) score was >22.0 (GRCh37, v1.6) [60], or if the variant was predicted “damaging” or “probably damaging” by SIFT [61] and PolyPhen2 [62]. Deletions are not shown as CADD scores cannot be calculated.

Figure 2. (A) Location of rare, predicted damaging MYH6 coding sequence variants by residue. Variants were considered rare if allele frequency was <1 × 10^{-3} in both the Genome Aggregation Database (gnomAD) Genomes dataset v3.1.2 and the Allele Frequency Aggregator (ALFA) dataset (release version 20201027095038). Single nucleotide variants were considered predicted damaging if the scaled Combined Annotation Dependent Depletion (CADD) score was >22.0 (GRCh37, v1.6) [60], or if the variant was predicted “damaging” or “probably damaging” by SIFT [61] and PolyPhen2 [62]. Deletions are not shown as CADD scores cannot be calculated. (B) Schematic of α-MHC domains. ELC, essential light chain; RLC, regulatory light chain; MyBP—C, myosin binding protein C; ACD, assembly competent domain.

4. Conclusions

HLHS is a complex and genetically heterogeneous disease, and the origins of HLHS are likely multigenic. Evidence suggests MYH6 variants are etiologic in a significant percentage of HLHS. Many of the studies cited in this paper identified additional candidate variants that may be contributing to cardiac disease development, including some patients carrying compound heterozygous MYH6 variants. New bioinformatic tools, such as Oligogenic Resource for Variant AnaLysis (ORVAL) [83], are designed to identify candidate pathogenic combinations of variants and are likely to be useful in elucidating the multigenic origins of HLHS and related disorders. The presence of additional genetic variants and environmental influences may explain some of the discrepancies between bioinformatic predictions of MYH6 variant pathogenicity and the reported cellular and clinical phenotypes. In any event, MYH6 variants will remain an important genetic risk factor for HLHS, having prognostic significance irrespective of other factors.

Our published work, [8,75] and many of the studies discussed in this paper, supports our hypothesis that atrial dysfunction due to sarcomere disorganization impairs atrial contractility during cardiac development leading to HLHS. These changes in atrial cardiomyocytes would likely impair atrial contractility in single ventricle patients postnatally, leading to heart failure over time. We anticipate that future longitudinal analyses will allow us to better understand the impact of MYH6 variants on long-term cardiac function in HLHS.
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Institutional Review Board Statement: This study was conducted in accordance with the principles outlined in the Declaration of Helsinki and institutionally approved research (IRB) protocols by Children’s Wisconsin (Milwaukee, WI, United States). Subjects were consented through the CHD Tissue Bank (IRB #CHW 06/229, GC 300) and the Wisconsin Pediatric Cardiac Registry (IRB #CHW 09/91, GC889), IRB-approved research databases housed at Children’s prior to inclusion in the study. All associated clinical outcome variables were obtained through these protocols.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Restrictions apply to the availability of these data. Data is restricted as it contains protected patient information.

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References

1. Tchervenkov, C.I.; Jacobs, M.L.; Tahta, S.A. Congenital Heart Surgery Nomenclature and Database Project: Hypoplastic left heart syndrome. Ann. Thorac. Surg. 2000, 69, S170–S179. [CrossRef]  
2. McBride, K.L.; Pignatelli, R.; Lewin, M.; Ho, T.; Fernbach, S.; Menesses, A.; Lam, W.; Leal, S.M.; Kaplan, N.; Schiekelman, P.; et al. Inheritance analysis of congenital left ventricular outflow tract obstruction malformations: Segregation, multiplex relative risk, and heritability. Am. J. Med. Genet. A 2005, 134A, 180–186. [CrossRef] [PubMed]  
3. Hinton, R.B., Jr.; Martin, L.J.; Tabangin, M.E.; Mazwi, M.L.; Cripe, L.H.; Benson, D.W. Hypoplastic left heart syndrome is heritable. J. Am. Coll. Cardiol. 2007, 50, 1590–1595. [CrossRef] [PubMed]  
4. Lara, D.A.; Ethen, M.K.; Canfield, M.A.; Nembhard, W.N.; Morris, S.A. A population-based analysis of mortality in patients with Turner syndrome and hypoplastic left heart syndrome using the Texas Birth Defects Registry. Congenit. Heart Dis. 2017, 12, 105–112. [CrossRef] [PubMed]  
5. Grossfeld, P.D.; Mattina, T.; Lai, Z.; Favier, R.; Jones, K.L.; Cotter, F.; Jones, C. The 11q terminal deletion disorder: A prospective study of 110 cases. Am. J. Med. Genet. A 2004, 129A, 51–61. [CrossRef] [PubMed]  
6. Liu, X.; Yagi, H.; Saeed, S.; Bais, A.S.; Gabriel, G.C.; Chen, Z.; Peterson, K.A.; Li, Y.; Schwartz, M.C.; Reynolds, W.T.; et al. The complex genetics of hypoplastic left heart syndrome. Nat. Genet. 2017, 49, 1152–1159. [CrossRef] [PubMed]  
7. Yagi, H.; Liu, X.; Gabriel, G.C.; Wu, Y.; Peterson, K.; Murray, S.A.; Aronow, B.J.; Martin, L.J.; Benson, D.W.; Lo, C.W. The Genetic Landscape of Hypoplastic Left Heart Syndrome. Pediatr. Cardiol. 2018, 39, 1069–1081. [CrossRef]  
8. Tomita-Mitchell, A.; Stamm, K.D.; Mahnke, D.K.; Kim, M.S.; Hidestrand, P.M.; Liang, H.L.; Goetsch, M.A.; Hidestrand, M.; Simpson, P.; Pelech, A.N.; et al. Impact of MYH6 variants in hypoplastic left heart syndrome. Physiol. Genom. 2016, 48, 912–921. [CrossRef]  
9. Theis, J.L.; Zimmermann, M.T.; Evans, J.M.; Ecklof, B.W.; Wieben, E.D.; Qureshi, M.Y.; O’Leary, P.W.; Olson, T.M. Recessive MYH6 Mutations in Hypoplastic Left Heart With Reduced Ejection Fraction. Circ. Cardiovasc. Genet. 2015, 8, 564–571. [CrossRef]  
10. Yang, C.; Xu, Y.; Yu, M.; Lee, D.; Alharti, S.; Hellen, N.; Ahmad Shaik, N.; Banaganapalli, B.; Sheikh Ali Mohamoud, H.; Elango, R.; et al. Induced pluripotent stem cell modelling of HLHS underlines the contribution of dysfunctional NOTCH signalling to impaired cardiogenesis. Hum. Mol. Genet. 2017, 26, 3031–3045. [CrossRef]
11. Elliott, D.A.; Kirk, E.P.; Yeoh, T.; Chandar, S.; McKenzie, F.; Taylor, P.; Grossfeld, P.; Fatkin, D.; Jones, O.; Hayes, P.; et al. Cardiac homeobox gene NKX2-5mutations and congenital heart disease. J. Am. Coll. Cardiol. 2003, 41, 2072–2076. [CrossRef]

12. McBride, K.L.; Zender, G.A.; Fitzgerald-Butt, S.M.; Seagraves, N.J.; Fernbach, S.D.; Zapata, G.; Lewin, M.; Towbin, J.A.; Belmont, J.W. Association of common variants in ERBB4 with congenital left ventricular outflow tract obstruction defects. Birth Defects Res. A Clin. Mol. Teratol. 2011, 91, 162–168. [CrossRef] [PubMed]

13. Reamon-Buettner, S.M.; Ciribilli, Y.; Inga, A.; Borlak, J. A loss-of-function mutation in the binding domain of HAND1 predicts hypoplasia of the human hearts. Hum. Mol. Genet. 2008, 17, 1397–1405. [CrossRef] [PubMed]

14. Dasgupta, C.; Martinez, A.M.; Zuppan, C.W.; Shah, M.M.; Bailey, L.L.; Fletcher, W.H. Identification of connexin43 (alpha1) gap junction gene mutations in patients with hypertrophic left heart syndrome by denaturing gradient gel electrophoresis (DGGE). Mutat. Res. 2001, 479, 173–186. [CrossRef]

15. Berdougo, E.; Coleman, H.; Lee, D.H.; Stainier, D.Y.; Yelon, D. Mutation of weak atrium/atrial myosin heavy chain disrupts atrial function and influences ventricular morphogenesis in zebrafish. Development 2003, 130, 6121–6129. [CrossRef] [PubMed]

16. Auman, H.J.; Coleman, H.; Riley, H.E.; Okale, F.; Tsai, H.J.; Yelon, D. Functional modulation of cardiac form through regionally confined cell shape changes. PLoS Biol. 2007, 5, e53. [CrossRef] [PubMed]

17. Abu-Daya, A.; Sater, A.K.; Wells, D.E.; Mohun, T.J.; Zimmerman, L.B. Absence of heartbeat in the Xenopus tropicalis mutation muzak is caused by a nonsense mutation in cardiac myosin myh6. Dev. Biol. 2009, 336, 20–29. [CrossRef]

18. Reiser, P.J.; Portman, M.A.; Ning, X.H.; Moravec, C.S. Human cardiac myosin heavy chain isoforms in fetal and failing adult atria and ventricles. Am. J. Physiol. Heart Circ. Physiol. 2001, 280, H1814–H1820. [CrossRef]

19. Wessels, A.; Vermeulen, J.L.M.; Viragh, S.Z.; Moorman, A.F.M. The ontogenesis of myosin heavy chain isoforms in the developing human heart. Ann. N. Y. Acad. Sci. 1990, 588, 461–464. [CrossRef]

20. Wessels, A.; Vermeulen, J.L.M.; Viragh, S.Z.; Kalmán, F.; Lamers, W.H.; Moorman, A.F.M. Spatial distribution of “tissue-specific” antigens in the developing human heart and skeletal muscle. II. An immunohistochemical analysis of myosin heavy chain isoform expression patterns in the embryonic heart. Anat. Rec. 1991, 229, 355–368. [CrossRef]

21. Solomon, S.D.; Geisterfer-Lowrance, A.; Joshs, H.P.; Gudrun, H.; Jarcho, J.A.; Morton, C.C.; McBride, W.O.; Mitchell, A.L.; Bale, A.E.; McKenna, W.; et al. A locus for familial hypertrophic cardiomyopathy is closely linked to the cardiac myosin heavy chain genes, CRI-L436, and CRI-L329 on chromosome 14 at q11-q12. Am. J. Hum. Genet. 1990, 47, 389–394. [PubMed]

22. Tanigawa, G.; Jarcho, J.A.; Kass, S.; Solomon, S.D.; Joshs, H.P.; Seidman, J.G.; Seidman, C.E. A molecular basis for familial hypertrophic cardiomyopathy: An alpha/beta cardiac myosin heavy chain hybrid gene. Cell Press 1990, 62, 991–998. [CrossRef]

23. Pulignani, S.; Vecoli, C.; Borghini, A.; Foffa, I.; Ait-Ali, L.; Andreassi, M.G. Targeted Next-Generation Sequencing in Patients with Non-syndromic Congenital Heart Disease. Pediatr. Cardiol. 2018, 39, 682–689. [CrossRef] [PubMed]

24. Priest, J.R.; Oseogawa, K.; Mohammed, N.; Nanda, V.; Kundu, R.; Schultz, K.; Lammer, E.J.; Girirajan, S.; Scheetz, T.; Waggott, D.; et al. De Novo and Rare Variants at Multiple Loci Support the Oligogenic Origins of Atrioventricular Septal Heart Defects. PLoS Genet. 2016, 12, e1005963. [CrossRef] [PubMed]

25. Jia, Y.; Louw, J.J.; Breckpot, J.; Callewaert, B.; Barrea, C.; Sznejar, Y.; Gewillig, M.; Souche, E.; Dehaspe, L.; Vermeesch, J.R.; et al. The diagnostic value of next generation sequencing in familial nonsyndromic congenital heart defects. Am. J. Med. Genet. A 2015, 167A, 1822–1829. [CrossRef]

26. Granados-Rieron, J.T.; Ghosh, T.K.; Pope, M.; Bu’Lock, F.; Thornborough, C.; Eason, J.; Kirk, E.P.; Fatkin, D.; Feneley, M.P.; Harvey, R.P.; et al. Alpha-cardiac myosin heavy chain (MYH6) mutations affecting myofibril formation are associated with congenital heart defects. Hum. Mol. Genet. 2009, 18, 4007–4016. [CrossRef]

27. Jin, S.C.; Homsey, J.; Zaidi, S.; Lu, Q.; Morton, S.; DePalma, S.R.; Zeng, X.; Qi, H.; Chang, W.; Sierant, M.C.; et al. Contribution of common variants in ERBB4 with congenital left ventricular outflow tract obstruction defects. Birth Defects Res. A Clin. Mol. Teratol. 2011, 91, 162–168. [CrossRef] [PubMed]

28. Bowles, N.E.; Jou, C.J.; Arrington, C.B.; Kennedy, B.J.; Earl, A.; Matsunami, N.; Meyers, L.L.; Etheridge, S.P.; Saarel, E.V.; Bleyl, S.B.; et al. Exome analysis of a family with Wolff-Parkinson-White syndrome identifies a novel disease locus. Am. J. Med. Genet. A 2015, 167A, 2975–2984. [CrossRef] [PubMed]

29. Liu, Y.; Cao, Y.; Li, Y.; Lei, D.; Li, L.; Hou, Z.L.; Han, S.; Meng, M.; Shi, J.; Zhang, Y.; et al. Novel Genetic Variants of Sporadic Atrial Septal Defect (ASD) in a Chinese Population Identified by Whole-Exome Sequencing (WES). J. Cardiovasc. Dev. Dis. 2022, 9, 141–145. [CrossRef] [PubMed]
77. Flashman, E.; Redwood, C.; Moolman-Smook, J.; Watkins, H. Cardiac myosin binding protein C: Its role in physiology and disease. *Circ. Res.* 2004, 94, 1279–1289. [CrossRef] [PubMed]

78. Homburger, J.R.; Green, E.M.; Caleshu, C.; Sunitha, M.S.; Taylor, R.E.; Ruppel, K.M.; Metpally, R.P.; Colan, S.D.; Michels, M.; Day, S.M.; et al. Multidimensional structure-function relationships in human beta-cardiac myosin from population-scale genetic variation. *Proc. Natl. Acad. Sci. USA* 2016, 113, 6701–6706. [CrossRef]

79. Kelly, M.A.; Caleshu, C.; Morales, A.; Buchan, J.; Wolf, Z.; Harrison, S.M.; Cook, S.; Dillon, M.W.; Garcia, J.; Haverfield, E.; et al. Adaptation and validation of the ACMG/AMP variant classification framework for MYH7-associated inherited cardiomyopathies: Recommendations by ClinGen’s Inherited Cardiomyopathy Expert Panel. *Genet. Med.* 2018, 20, 351–359. [CrossRef]

80. Nag, S.; Trivedi, D.V.; Sarkar, S.S.; Adhikari, A.S.; Sunitha, M.S.; Sutton, S.; Ruppel, K.M.; Spudich, J.A. The myosin mesa and the basis of hypercontractility caused by hypertrophic cardiomyopathy mutations. *Nat. Struct. Mol. Biol.* 2017, 24, 525–533. [CrossRef]

81. Spudich, J.A. The myosin mesa and a possible unifying hypothesis for the molecular basis of human hypertrophic cardiomyopathy. *Biochem. Soc. Trans.* 2015, 43, 64–72. [CrossRef] [PubMed]

82. Colegrave, M.; Peckham, M. Structural implications of beta-cardiac myosin heavy chain mutations in human disease. *Anat. Rec.* 2014, 297, 1670–1680. [CrossRef] [PubMed]

83. Renaux, A.; Papadimitriou, S.; Versbraegen, N.; Nachtegaele, C.; Boutry, S.; Nowe, A.; Smits, G.; Lenaerts, T. ORVAL: A novel platform for the prediction and exploration of disease-causing oligogenic variant combinations. *Nucleic Acids Res.* 2019, 47, W93–W98. [CrossRef] [PubMed]