

Original Research

A New Leu714Arg Variant in the Converter Domain of *MYH7* is Associated with a Severe Form of Familial Hypertrophic Cardiomyopathy

Maria V. Golubenko^{1,*}, Elena N. Pavlyukova², Ramil R. Salakhov¹,
Oksana A. Makeeva¹, Konstantin V. Puzyrev², Oleg S. Glotov³, Valery P. Puzyrev¹,
Maria S. Nazarenko¹

¹Research Institute of Medical Genetics, Tomsk National Research Medical Center, Russian Academy of Sciences, 634050 Tomsk, Russia

²Cardiology Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, 634050 Tomsk, Russia

³Department of Genomic Medicine, D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology, 199034 Saint-Petersburg, Russia

*Correspondence: maria.golubenko@medgenetics.ru (Maria V. Golubenko)

Academic Editor: Maria V. Golubenko

Submitted: 1 October 2023 Revised: 15 January 2024 Accepted: 29 January 2024 Published: 23 February 2024

Abstract

Background: Hypertrophic cardiomyopathy is the most frequent autosomal dominant disease, yet due to genetic heterogeneity, incomplete penetrance, and phenotype variability, the prognosis of the disease course in pathogenic variant carriers remains an issue. Identifying common patterns among the effects of different genetic variants is important. **Methods:** We investigated the cause of familial hypertrophic cardiomyopathy (HCM) in a family with two patients suffering from a particularly severe disease. Searching for the genetic variants in HCM genes was performed using different sequencing methods. **Results:** A new missense variant, p.Leu714Arg, was identified in exon 19 of the beta-myosin heavy chain gene (*MYH7*). The mutation was found in a region that encodes the ‘converter domain’ in the globular myosin head. This domain is essential for the conformational change of myosin during ATP cleavage and contraction cycle. Most reports on different mutations in this region describe severe phenotypic consequences. The two patients with the p.Leu714Arg mutation had heart failure early in life and died from HCM complications. **Conclusions:** This case presents a new likely pathogenic variant in *MYH7* and supports the hypothesis that myosin converter mutations constitute a subclass of HCM mutations with a poor prognosis for the patient.

Keywords: hypertrophic cardiomyopathy; *MYH7*; myosin converter domain

1. Introduction

Hypertrophic cardiomyopathy (HCM) is a primary myocardium disease characterized by an asymmetric increase in heart muscle mass, predominantly in the interventricular septum, hypertrophied cardiocytes, cellular and sarcomeric disarray, and fibrosis [1]. Major functional impairments are compromised relaxation and hypercontractility [2]. In most cases, the disease has a genetic cause that leads to an autosomal dominant trait being transmitted throughout families. Occasionally, a *de novo* variant may be responsible for the phenotype in previously unaffected families. Significant contributors to the disease are mutations in the beta-myosin heavy chain (*MYH7*) and the cardiac isoforms of myosin binding protein-C (*MYBPC3*). These two genes may account for about two-thirds of all genetically confirmed cases [3]. Most genes that possess pathogenic variants code for proteins constituting the sarcomere or contributing to controlling contraction.

The heterogeneity of inherited causes of HCM corresponds to a broad spectrum of highly variable phenotypes. Mild cases with onset of symptoms in the fourth decade of life or even later contrast with early onset, severe symp-

toms, and a high risk of sudden cardiac death. The underlying genetic defect can partly explain the variability in the phenotype; however, genetic modifiers and polymorphisms may modulate the functional consequences of the mutation [4]. Thus, it is frequently difficult to predict the clinical outcome if a diagnosis has been established early or before the symptomatic stage. Therefore, familial case studies are frequently useful to improve our understanding of the biological consequences of a genetic alteration. Here, we document the new likely pathogenic variant together with its clinical phenotype and discuss the functional implications that this variant could have.

2. Materials and Methods

2.1 Clinical Evaluation

The initial (tentative) diagnosis of HCM in the proband was performed in an army hospital during military service. A son was first diagnosed as having a congenital atrial septal defect following surgical correction of that defect. Treatment and follow-up investigations of both patients were conducted in the Cardiology Research Institute



of Tomsk National Research Medical Center. The diagnosis of hypertrophic cardiomyopathy was based on the evaluation of symptoms, 12-lead ECG monitoring at rest and during exercise, X-ray studies, 2D- and Doppler echocardiography, and quantitative coronary angiography with intracardiac blood pressure measurement. Informed consent for the genetic analysis was obtained from the patients and unaffected relatives.

2.2 Genetic Analysis

For three family members (two affected, one unaffected), genomic DNA was extracted from venous EDTA blood samples using phenol–chloroform extraction. The genes coding for beta-myosin heavy chain (*MYH7*) and cardiac myosin binding protein C (*MYBPC3*) were investigated as candidate genes by analyzing single-strand conformation polymorphism (SSCP) using gel migration behavior of the amplified exons of the two genes. For PCR amplification, PCR primers were designed based on reference gene sequences using Primer3 v 4.1.0 software [5]. For SSCP analysis, non-denaturing polyacrylamide gradient gels (5 to 17% acrylamide and 0 to 0.5% sucrose) were used. Electrophoresis was performed in horizontal gel chambers at two temperatures (12 °C and 24 °C). DNA was visualized by silver staining. Sanger sequencing of PCR products with altered SSCP patterns was performed in both directions by the ABI3730 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) using PCR primers. Additional screening was conducted for the proband using NGS (TruSight™ Cardiomyopathy Sequencing Panel, Illumina, San Diego, CA, USA) to prevent additional clinically relevant genetic variants.

Initial SSCP screening and sequencing were performed at the Max Planck Institute for Physiological and Clinical Research in Bad Nauheim, Germany. Sample preparation and confirmation sequencing were conducted in the Institute of Medical Genetics using the Core Facility «Medical Genomics» equipment of Tomsk NRMC. In particular, DNA sequencing was performed on the ABI3730 Genetic Analyzer. NGS was performed on a MiSeq instrument (Illumina, Inc., San Diego, CA, USA) according to the TruSight™ Cardiomyopathy Sequencing Panel protocol in the Clinical Genetic Research department of the City Hospital No. 40, St. Petersburg, Russia.

The Local Biomedical Ethics Committee approved the study at the Research Institute of Medical Genetics and the Cardiology Research Institute, Tomsk National Research Medical Center. The DNA samples were stored in the collection of samples “Biobank of the North Eurasia population” in the Research Institute of Medical Genetics, Tomsk NRMC.

3. Results

3.1 Clinical Features

A small Tomsk (Russia) family with two seriously affected patients (father and son) was studied. The pedigree of the family is shown in Fig. 1. The proband (father, II-2) had one unaffected brother (II-1) and one affected child (III-1). The parents of the proband died from unidentified causes (no further information available). Cardiac disease was first recognized in the proband upon hospitalization at the age of 22 years. Reduced exercise tolerance, dyspnea, extrasystoles, myocardial hypercontractility, and an ECG suggestive of a cardiomyopathy in the absence of systemic conditions able to explain these features (e.g., arterial hypertension) led tentatively to diagnosing hypertrophic cardiomyopathy.

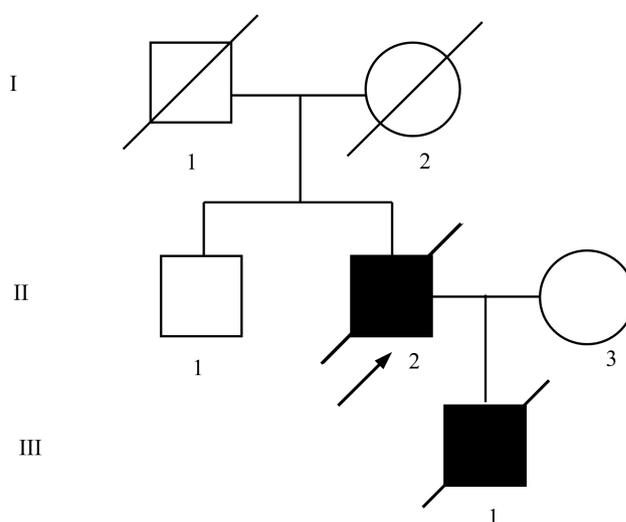


Fig. 1. Pedigree of the family. Squares and circles indicate male and female members of the family, respectively. Slashed symbols designate deceased members. Filled and open symbols refer to affected and unaffected members, respectively. The clinical status of the grandparents (generation I) was unassessed. The arrow points to the proband.

The clinical status worsened gradually in subsequent years. At 30 years, thickening of the interventricular septum was apparent (see Fig. 2A; documented at 37 years), together with mild mitral regurgitation. Severe arrhythmia (atrial flutter and frequent extrasystoles), X-ray evidence of pulmonary hypertension, impaired ventricular contractility and relaxation, and dilatation of the left atrium and ventricle were indicative of advanced cardiac failure (New York Heart Association (NYHA) stage III). The absence of other vascular or systemic malfunctions supported HCM as the primary diagnosis. The disease was complicated by the development of exudative pericarditis (Fig. 2A). Medication (angiotensin converting enzyme (ACE) inhibitors, cardiac glycosides, diuretics) was of limited use. At the age of

42 years, the patient had dyspnea at rest, severely impaired systolic and diastolic function, an ejection fraction of 40%, atrial fibrillation, and severe complications by pulmonary hypertension and pericarditis, indicating that the patient's condition was close to end-stage heart failure (NYHA stage III-IV). The patient died soon after.

The son suffered from early childhood onwards. His case was confounded by an atrial septal defect diagnosed at the age of 2.5 years and corrected by surgery at the age of 6. Even before surgery, conspicuous interventricular septum hypertrophy became apparent. At the age of 17, his condition was characterized by grossly reduced exercise tolerance, dyspnea at rest, hypertrophy of the posterior wall (14 mm) in addition to the septum (23 mm, see Fig. 2B), impaired cardiac performance (ejection fraction at rest 61%) and cardiomegaly (based on chest X-ray and an index of LV myocardial mass of 205.6 g/m²). The son also exhibited signs of pulmonary hypertension. Therapy using calcium antagonists and beta-blockers was ineffective or even worsened the condition. At the age of 18, the son died of sudden cardiac death provoked by physical efforts. In summary, both patients severely suffered from cardiac disease, which started early in life. The brother of the proband (see Fig. 1, II-1) had typical echocardiographic characteristics and no clinical symptoms. The proband's wife (Fig. 1, II-3) had no symptoms and was not clinically evaluated.

3.2 Genetic Analysis

Two genes (*MYH7* and *MYBPC3*), whose mutations account for about 50% of all familial cases of HCM, were screened in the patients using the SSCP method. PCR products containing exon sequences in the myosin binding protein C gene did not show differences in SSCP patterns obtained from the DNA of the patients, while some SNPs were observed (not shown). Among 40 *MYH7* exons tested, a unique altered SSCP pattern was identified for exon 19. Sanger sequencing of this exon revealed T to G transversion (A to C transversion in the genomic sequence) in the second position of codon 714 leading to a Leu to Arg substitution: *MYH7*(NM_000257.4):c.2141T>G, m.14:23895194-A-C (GRCh37/hg19), or m.14:23425985-A-C (GRCh38/hg38). The substitution was identified in both patients but not in the unaffected brother of the proband. This variant is novel and is absent in population and mutation genomic databases (ClinVar, GnomAD, RuSeq) [6–8].

The mutation introduces a positive charge in a normally hydrophobic position located in the region of the so-called converter domain of the protein that contributes to the movement of the myosin head during contraction [9]. According to the ACMG/AMP guidelines for the interpretation of sequence variants [10], this new variant meets criteria PM1, PM2, PP2, PP3, and PP4 and can be classified as likely pathogenic, according to the rule “2 Moderate (PM1–PM6) and ≥2 Supporting (PP1–PP5) criteria” (see Tables

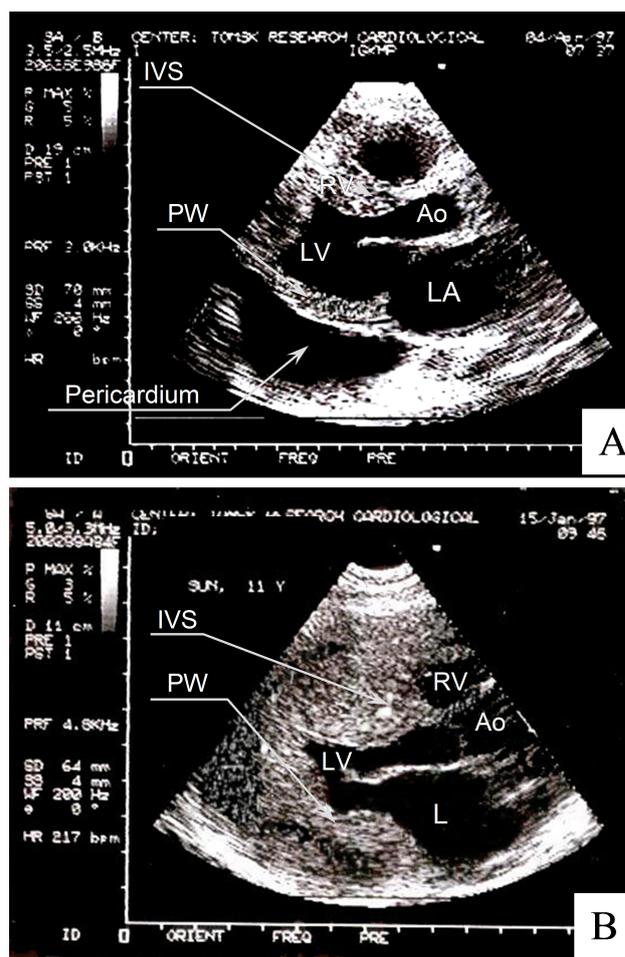


Fig. 2. Echocardiographic investigation of the two patients in the family. (A) Father. (B) Son. IVS, interventricular septum; PW, left ventricular posterior wall; LV and RV, left and right ventricle; LA, left atrium; Ao, aorta. The arrow indicates pericardial space filled with exudative fluid in panel A.

4, 5 in [10]). Online resource VarSome [11] also designates this variant as likely pathogenic. Hence, we suppose that this new variant is the cause of the observed hypertrophic cardiomyopathy in this family.

The severe course of the disease suggested a contribution by additional genetic variants because patients with two pathogenic variants usually have a “malignant” phenotype [3]. To identify/exclude additional mutations in the patient, we performed sequencing coding regions of 46 cardiomyopathy genes with TruSight™ Cardiomyopathy Sequencing Panel, Illumina, for the DNA of the proband (Fig. 1, II-2). The results confirmed a p.Leu714Arg variant in *MYH7*. No additional pathogenic/likely pathogenic/uncertain significance variants were identified. No additional genetic tests were conducted for the son (Fig. 1, III-1).

4. Discussion

We have identified a novel *MYH7* variant in two patients from a nuclear family, a father and son, who were

both affected by hypertrophic cardiomyopathy and cardiac failure. The mutation was a c.2141T>G transversion, resulting in the replacement of leucine by arginine in codon 714. The variant was found in the two patients but not in the unaffected brother of the proband. Since both patients had early onset variations of the disease, relatively fast progression of symptoms, and premature cardiac death, the cardiomyopathy course in this family can be described as “malignant”.

However, the contribution of the mutation to the phenotype cannot easily be assessed because both patients had additional conditions not generally associated with HCM. The father was suffering from recurrent pericarditis for many years with apparently serious consequences for cardiac performance. Volume overload caused by or associated with HCM and pulmonary hypertension presumably contributed to a vicious cycle resulting in end-stage failure. The son was born with a congenital defect, which led to hemodynamic consequences. To summarize the clinical evaluation, these patients were exposed not only to HCM but to confounding conditions, adding to the myocardial dysfunction caused by the myosin heavy chain mutation. However, the uncommonly severe phenotype in both patients is a solid reason to argue for a severe characterization of the 714 mutation. Sometimes, the severe disease course may be explained by more than one pathogenic variant in the family or additional variants in other genes that are not pathogenic but have some modifying effect [3,12]. However, sequencing of the coding regions for 46 cardiomyopathy-associated genes in the proband did not find candidate variants with likely functional effects. It should be noted that the possible genetic cause for the congenital heart defect in the son was not investigated.

Exon 19 *MYH7* contributes to the part of the C-terminal end of the myosin head known as the “converter domain” [13]. This region consists of a stretch of 68 amino acids (in the human beta-myosin chain residues 709 to 777) thought to serve as a socket for the adjacent (C-terminal) alpha-helical extension (or “neck”) stabilized by two light chains. It has been concluded from the model building as well as from functional assays that the “neck” is in the correct orientation and position to act as a lever (for review and references, see [14]). The converter contributes to the “power stroke” of the contractile cycle by, presumably, transducing a conformational change resulting from ATP cleavage in the globular myosin head to the alpha-helical “lever arm” extending from the C-terminus of the head. The position between the myosin head and the lever arm may function as a “relay station” between the ATP cleavage center and the lever arm. Internal elastic distortions of the converter domain may be how the converter mediates a reorientation (“swinging”) of the lever arm. That swinging contributes to the translocation of actin along myosin filaments. The function of the lever in terms of speed of actin transport promoted by myosin heads depends on the length of the

neck. The addition of light chain binding sites increased the speed of actin movement *in vitro*, and truncations led to a decrease, as was shown by exposing actin filaments to myosin heads of *Dictyostelium discoideum* immobilized on a solid surface [15]. Altered functions and pleiotropic effects, including changes in ATPase activity patterns, have also been demonstrated with truncated converter regions of myosin II from *Dictyostelium discoideum* [16]. These experiments strongly suggest that the converter region and the adjacent lever are essential for the motor system. Recently, it has been shown that conformational change in the converter is integral to the mechanochemical coupling of myosin, particularly for the ADP release step [17].

A query to the ClinVar database [6] (accessed September 2023) provided information on more than 90 different amino acid variants in 48 positions within the converter region (71% of the converter amino acids), classified from uncertain significance to pathogenic. In the first 16 amino acids (709–726), at least one missense variant was described in each position (except 709 and 711), and altogether with p.Leu714Arg, there are 33 different amino acid variations in patients for these 18 positions (Fig. 3A, Ref. [6,18–20]). It means that this converter region is frequently mutated in cardiomyopathies. Indeed, analysis of 2913 patients with HCM allowed us to identify significant enrichment of disease-associated variants in the converter, with earlier disease onset in the carriers of these variants [18]. According to the summary of published and own data provided in [18], among 526 patients with 25 different mutations in the converter, 407 had HCM (77%), the mean age of diagnosis was 8 years, and 97% of the variant carriers were symptomatic at the age of 35; in addition, 184 patients (37%) had either sudden cardiac death or cardiac transplantation [19]. A recent study showed that variants in the converter domain have significantly higher penetrance than variants in other MYH regions [20].

Some experimental studies analyzed the consequences of mutations in the converter. For instance, using isolated muscle fibers (obtained from m. soleus of an HCM patient suffering from a p.Arg719Trp mutation), increased generation of force, and fiber stiffness were demonstrated as a consequence of the Arg to Trp exchange [23]. M. soleus expresses the same beta-myosin isoform as the myocardium. Stiffness under rigorous conditions and during relaxation and force development increased by similar amounts (about 50%). Increased fiber stiffness would imply a reduced ability by the myosin molecule to switch back to the original conformation at the end of one power stroke. The authors suggested that the converter is a sub-domain in myosin within which the transient structural change is needed to drive the action of the lever arm [23]. Further studies demonstrated that the p.Arg723Gly variant has a similar effect on the fiber stiffness, whereas p.Ile736Thr has essentially no effect [24,25]. Other investigators showed slightly decreased contractile force but 15% faster velocity

not change the molecular charge. It might be proposed that clinically severe phenotypes mark the majority of converter mutations in the region at or near 714, with a high risk of life-threatening complications. Using epidemiological inference, published evidence suggests a common or at least related mechanism for these mutations. Based on this suggestion, we propose that the p.Leu714Arg mutation is responsible for a similarly severe dysfunction in the converter domain as the p.Gly716Arg mutation.

5. Conclusions

A new likely pathogenic variant in *MYH7* (NM_000257.4):c.2141T>G leading to amino acid replacement p.Leu714Arg in the cardiac beta-myosin heavy chain has been identified in two related patients suffering from “malignant” hypertrophic cardiomyopathy. Analysis of the mutation spectrum in this gene showed that the substitution is located in the critical functional converter domain, marked by a high rate of functionally relevant variants. In most cases, mutations in the converter myosin domain lead to early-onset hypertrophic cardiomyopathy with a high risk of life-threatening complications.

Availability of Data and Materials

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study.

Author Contributions

MVG designed and performed the research, analysed the data, wrote the manuscript. ENP and KVP performed diagnostics and observation of the patients, collected clinical data, prepared and discussed clinical section of the manuscript. OAM participated conducting the experiments, and discussing the results. RRS and OSG performed NGS experiments and interpreted the results. VPP and MSN planned and designed the research, discussed and edited the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Informed consent was obtained from the participants of the study. The study was conducted in accordance with the Declaration of Helsinki and approved by the Local Biomedical Ethics Committee at the Research Institute of Medical Genetics, Tomsk National Research Medical Center (protocol # 10, February 15, 2021) and the Local Biomedical Ethics Committee at the Cardiology Research Institute Tomsk National Research Medical Center (protocol # 151, December 22, 2016).

Acknowledgment

The authors dedicate this article to the memory of Professor Hans-Peter Vosberg, who made a significant contribution to the genetic studies of hypertrophic cardiomyopathy and who participated in writing the first version of the manuscript.

Funding

The study was performed as a part of basic research program of the Institute of Medical Genetics, Tomsk NRMC. M.V.G. received a postdoc fellowship of Max-Planck Society (Germany).

Conflict of Interest

The authors declare no conflict of interest.

References

- [1] Vosberg HV, McKenna WJ. Cardiomyopathies. In Rimoin DL, Connor JM, Pyeritz RE Korf BR (eds.) Principles and Practice of Medical Genetics (pp. 1342–1416). Churchill Livingstone: London, Edinburgh, New York. 2002.
- [2] Yotti R, Seidman CE, Seidman JG. Advances in the Genetic Basis and Pathogenesis of Sarcomere Cardiomyopathies. Annual Review of Genomics and Human Genetics. 2019; 20: 129–153.
- [3] Marian AJ. Molecular Genetic Basis of Hypertrophic Cardiomyopathy. Circulation Research. 2021; 128: 1533–1553.
- [4] Harper AR, Goel A, Grace C, Thomson KL, Petersen SE, Xu X, *et al.* Common genetic variants and modifiable risk factors underpin hypertrophic cardiomyopathy susceptibility and expressivity. Nature Genetics. 2021; 53: 135–142.
- [5] Rozen S, Skaletsky H. Primer3 on the WWW for general users and for biologist programmers. Methods in Molecular Biology (Clifton, N.J.). 2000; 132: 365–386.
- [6] Landrum MJ, Kattman BL. ClinVar at five years: Delivering on the promise. Human Mutation. 2018; 39: 1623–1630.
- [7] Chen S, Francioli LC, Goodrich JK, Collins RL, Wang Q, Alfoldi J, *et al.* A genome-wide mutational constraint map quantified from variation in 76,156 human genomes. BioRxiv. (preprint)
- [8] Barbitoff YA, Khmelkova DN, Pomerantseva EA, Slepchenkov AV, Zubashenko NA, Mironova IV, *et al.* Expanding the Russian allele frequency reference via cross-laboratory data integration: insights from 7,452 exome samples. MedRxiv. (preprint)
- [9] Holmes KC. Muscle proteins—their actions and interactions. Current Opinion in Structural Biology. 1996; 6: 781–789.
- [10] Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, *et al.* Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genetics in Medicine: Official Journal of the American College of Medical Genetics. 2015; 17: 405–424.
- [11] Kopanos C, Tsiolkas V, Kouris A, Chapple CE, Albarca Aguilera M, Meyer R, *et al.* VarSome: the human genomic variant search engine. Bioinformatics (Oxford, England). 2019; 35: 1978–1980.
- [12] Glotov OS, Chernov AN, Glotov AS. Human Exome Sequencing and Prospects for Predictive Medicine: Analysis of International Data and Own Experience. Journal of Personalized Medicine. 2023; 13: 1236.
- [13] Houdusse A, Cohen C. Structure of the regulatory domain of scallop myosin at 2 Å resolution: implications for regulation. Structure (London, England: 1993). 1996; 4: 21–32.

- [14] Holmes KC. The swinging lever-arm hypothesis of muscle contraction. *Current Biology*. 1997; 7: R112–R118.
- [15] Uyeda TQ, Abramson PD, Spudich JA. The neck region of the myosin motor domain acts as a lever arm to generate movement. *Proceedings of the National Academy of Sciences of the United States of America*. 1996; 93: 4459–4464.
- [16] Kurzawa SE, Manstein DJ, Geeves MA. Dictyostelium discoideum myosin II: characterization of functional myosin motor fragments. *Biochemistry*. 1997; 36: 317–323.
- [17] Doran MH, Rynkiewicz MJ, Rasicci D, Bodt SML, Barry ME, Bullitt E, *et al.* Conformational changes linked to ADP release from human cardiac myosin bound to actin-tropomyosin. *The Journal of General Physiology*. 2023; 155: e202213267.
- [18] Homburger JR, Green EM, Caleshu C, Sunitha MS, Taylor RE, Ruppel KM, *et al.* Multidimensional structure-function relationships in human β -cardiac myosin from population-scale genetic variation. *Proceedings of the National Academy of Sciences of the United States of America*. 2016; 113: 6701–6706.
- [19] García-Gustiniani D, Arad M, Ortíz-Genga M, Barriales-Villa R, Fernández X, Rodríguez-García I, *et al.* Phenotype and prognostic correlations of the converter region mutations affecting the β myosin heavy chain. *Heart (British Cardiac Society)*. 2015; 101: 1047–1053.
- [20] Jansen M, de Brouwer R, Hassanzada F, Schoemaker AE, Schmidt AF, Kooijman-Reumerman MD, *et al.* Penetrance and Prognosis of MYH7 Variant-Associated Cardiomyopathies: Results From a Dutch Multicenter Cohort Study. *JACC. Heart Failure*. 2024; 12: 134–147.
- [21] Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, *et al.* The Protein Data Bank. *Nucleic Acids Research*. 2000; 28: 235–242.
- [22] Sehnal D, Bittrich S, Deshpande M, Svobodová R, Berka K, Bazgier V, *et al.* Mol* Viewer: modern web app for 3D visualization and analysis of large biomolecular structures. *Nucleic Acids Research*. 2021; 49: W431–W437.
- [23] Köhler J, Winkler G, Schulte I, Scholz T, McKenna W, Brenner B, *et al.* Mutation of the myosin converter domain alters cross-bridge elasticity. *Proceedings of the National Academy of Sciences of the United States of America*. 2002; 99: 3557–3562.
- [24] Seeböhm B, Matinmehr F, Köhler J, Francino A, Navarro-López F, Perrot A, *et al.* Cardiomyopathy mutations reveal variable region of myosin converter as major element of cross-bridge compliance. *Biophysical Journal*. 2009; 97: 806–824.
- [25] Brenner B, Seeböhm B, Tripathi S, Montag J, Kraft T. Familial hypertrophic cardiomyopathy: functional variance among individual cardiomyocytes as a trigger of FHC-phenotype development. *Frontiers in Physiology*. 2014; 5: 392.
- [26] Kronert WA, Melkani GC, Melkani A, Bernstein SI. A Failure to Communicate: MYOSIN RESIDUES INVOLVED IN HYPERTROPHIC CARDIOMYOPATHY AFFECT INTER-DOMAIN INTERACTION. *The Journal of Biological Chemistry*. 2015; 290: 29270–29280.
- [27] Anan R, Greve G, Thierfelder L, Watkins H, McKenna WJ, Solomon S, *et al.* Prognostic implications of novel beta cardiac myosin heavy chain gene mutations that cause familial hypertrophic cardiomyopathy. *The Journal of Clinical Investigation*. 1994; 93: 280–285.
- [28] Hwang TH, Lee WH, Kimura A, Satoh M, Nakamura T, Kim MK, *et al.* Early expression of a malignant phenotype of familial hypertrophic cardiomyopathy associated with a Gly716Arg myosin heavy chain mutation in a Korean family. *The American Journal of Cardiology*. 1998; 82: 1509–1513.
- [29] Ackerman MJ, VanDriest SL, Ommen SR, Will ML, Nishimura RA, Tajik AJ, *et al.* Prevalence and age-dependence of malignant mutations in the beta-myosin heavy chain and troponin T genes in hypertrophic cardiomyopathy: a comprehensive outpatient perspective. *Journal of the American College of Cardiology*. 2002; 39: 2042–2048.
- [30] Rai TS, Ahmad S, Bahl A, Ahuja M, Ahluwalia TS, Singh B, *et al.* Genotype phenotype correlations of cardiac beta-myosin heavy chain mutations in Indian patients with hypertrophic and dilated cardiomyopathy. *Molecular and Cellular Biochemistry*. 2009; 321: 189–196.
- [31] Zhao P, Cui HL, He TT, Wang JG, Wang D, Feng XX, *et al.* Familial hypertrophic cardiomyopathy caused by a de novo Gly716Arg mutation of the β -myosin heavy chain. *Cardiology in the Young*. 2017; 27: 467–472.
- [32] Walsh R, Thomson KL, Ware JS, Funke BH, Woodley J, McGuire KJ, *et al.* Reassessment of Mendelian gene pathogenicity using 7,855 cardiomyopathy cases and 60,706 reference samples. *Genetics in Medicine: Official Journal of the American College of Medical Genetics*. 2017; 19: 192–203.