Recessive MYH6 Mutations in Hypoplastic Left Heart With Reduced Ejection Fraction

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Background—The molecular underpinnings of hypoplastic left heart are poorly understood. Staged surgical palliation has dramatically improved survival, yet eventual failure of the systemic right ventricle necessitates cardiac transplantation in a subset of patients. We sought to identify genetic determinants of hypoplastic left heart with latent right ventricular dysfunction in individuals with a Fontan circulation.

Methods and Results—Evaluation of cardiac structure and function by echocardiography in patients with hypoplastic left heart and their first-degree relatives identified 5 individuals with right ventricular ejection fraction ≤40% after Fontan operation. Whole genome sequencing was performed on DNA from 21 family members, filtering for genetic variants with allele frequency <1% predicted to alter protein structure or expression. Secondary family-based filtering for de novo and recessive variants revealed rare inherited missense mutations on both paternal and maternal alleles of MYH6, encoding myosin heavy chain 6, in 2 patients who developed right ventricular dysfunction 3 to 11 years postoperatively. Parents and siblings who were heterozygous carriers had normal echocardiograms. Protein modeling of the 4 highly conserved amino acid substitutions, residing in both head and tail domains, predicted perturbation of protein structure and function.

Conclusions—In contrast to dominant MYH6 mutations with variable penetrance identified in other congenital heart defects and dilated cardiomyopathy, this study reveals compound heterozygosity for recessive MYH6 mutations in patients with hypoplastic left heart and reduced systemic right ventricular ejection fraction. These findings implicate a shared molecular basis for the developmental arrest and latent myopathy of left and right ventricles, respectively. 

Key Words: genomics ▶ heart defects, congenital ▶ heart failure ▶ hypoplastic left heart syndrome ▶ missense mutations ▶ myosin heavy chains

H ypoplastic left heart (HLH) is a severe congenital heart defect whose molecular pathogenesis and determinants of long-term outcome after Fontan palliation are poorly understood. HLH typically occurs as a sporadic, apparently non-Mendelian disorder. However, transmission of a dominant gene mutation may be constrained by embryonic lethality and lack of reproductive fitness in patients who reach childbearing age. Moreover, de novo or recessive mutations could underlie HLH in the absence of a family history of congenital heart disease (CHD). Genetic underpinnings of HLH are implicated by recurrence risks of 2% to 4% in families with 1 affected child and 25% in families with 2 affected children.1 Screening echocardiography in first-degree relatives has in fact identified less severe, often asymptomatic and undiagnosed, left-sided cardiac malformations including bicuspid/stenotic aortic valve, hypoplastic aortic arch, and coarctation of the aorta.2-4 Collectively, these findings could be explained by inheritance of mutations with incomplete penetrance and variable expression in ≥1 genes. Nonparametric genome-wide linkage analyses have identified several candidate chromosomal loci for HLH and implicate genetic heterogeneity.1 However, the hundreds of positional candidate genes within these loci pose a significant challenge for discovery of HLH susceptibility mutations. Nonpositional candidate gene strategies have revealed rare HLH-associated mutations in ZIC3,6 NOTCH1,7,8 and NKX2.5.9 Cytogenetic analyses have identified aneuploidy, including trisomy 13, trisomy 18, monosomy X (Turner syndrome), and 11qter monosomy (Jacobsen Syndrome) as potential rare causes for HLH associated with extracardiac syndromes.10,11

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Here, we used whole genome sequencing (WGS) in HLH probands and phenotypically characterized family members to overcome existing barriers to HLH gene discovery. This
family-based approach enabled comprehensive, hypothesis-free scanning of genomes and modeling for plausible modes of inheritance. We investigated individuals with HLH who developed impaired right ventricular systolic function after successful Fontan palliation and may be destined for cardiac transplantation.

Methods

Study Subjects
Probands with HLH and their first-degree relatives provided written informed consent under a research protocol approved by the Mayo Clinic Institutional Review Board. All family members were of European ancestry by self-report. Cardiac structure and function were assessed by 2-dimensional, M-mode, and Doppler echocardiography in probands and relatives. In addition, strain rate imaging and circulating NT-pro-B-type natriuretic peptide measurement were performed in probands. Genomic DNA was isolated from peripheral-blood white cells or saliva.

Genomic and Bioinformatics Analyses
Array comparative genomic hybridization using a custom 180K oligonucleotide microarray (Agilent, Santa Clara, CA) was performed on probands with a genome-wide functional resolution of ≈100 kilobases. Deletions >200 kilobases and duplications >500 kilobases were reported as clinically relevant. WGS and variant call annotation were performed on genomic DNA samples from 21 individuals from 5 families, using the Mayo Clinic Medical Genome Facility and Bioinformatics Core (Methods are provided in the Data Supplement). Variant call format files with single nucleotide variant and insertion/deletion calls from each individual were uploaded and analyzed using Ingenuity Variant Analysis software (QIAGEN, Redwood City, CA) where variants were functionally annotated and filtered by an iterative process. First, common variants were removed based on >1% frequency in ≥1 publicly available whole exome sequencing or WGS database: the Exome Variant Server (whole exome sequencing data from 6503 individuals),12 1000 Genomes (WGS data from 1092 individuals),11 and Complete Genomics Genome (WGS data from 69 individuals).14 Next, variants were excluded if they were present in >5 in-house WGS or whole exome sequencing data sets collected from 147 individuals not affected with HLH (false-positive filter) or identified within the top 1% most exonically variable genes. Functional variants were restricted to those that affected a protein sequence, canonical splice site, microRNA coding sequence/binding site, enhancer region, or transcription factor binding site within a promoter validated by The Encyclopedia of DNA Elements (ENCODE) chromatin-immunoprecipitation experiments.15 Using parental and sibling WGS data, rare, functional variants in each proband were then filtered for those that arose de novo or fit homozygous recessive, compound heterozygous, and X-linked recessive (males) modes of inheritance. The candidacy of inherited genes was further assessed by the presence in ≥2 cases and established associations with CHD (275 genes) or dilated cardiomyopathy (DCM)/heart failure (599 genes), based on the Ingenuity Variant Analysis software Knowledge Base, a manually curated resource that integrates data from top-tier scientific literature.

In Silico Protein Modeling
Structural models were generated from the Protein Data Bank,16 coordinates or homology models using Swiss-Model.17 The sequence represented by UniProt ID P13533 (version 152, July 9, 2014) was used in all MYH6 models. Images were generated with PyMOL18 and Visual Molecular Dynamics.19 Molecular dynamics trajectories were computed using Nanoscale Molecular Dynamics (NAMD).20 Chemistry at Harvard Macromolecular Mechanics22+Connectivity Map (CHARMM22+CMAP) force field21 and the Generalized Born Implicit Solvent (GBIS) model. Analyses were performed using custom scripts for Visual Molecular Dynamics and the R programming language (further details of computational methods are provided in the Data Supplement).

Results

Patients With HLH and Latent Right Ventricular Systolic Dysfunction
We identified 5 individuals with HLH who were palliated with a Fontan circulation and developed right ventricular ejection fraction ≤40% during the ensuing 2 to 22 years (Table). Four were in normal sinus rhythm and 1 had dual chamber epicardial pacing. None were on antiarrhythmic therapy. Symptoms of heart failure were minimal or absent at the time of study enrollment while on pharmacological therapy that included an angiotensin-converting enzyme inhibitor in all and variable combinations of digoxin, carvedilol, spironolactone, and furosemide. Four had increased NT-pro-B-type natriuretic peptide levels. Screening echocardiograms in the 10 parents (aged 34–50 years) and 6 siblings (aged 7–20 years) revealed no evidence for structural or myopathic heart disease (Figure 1).

WGS Reveals Recessive MYH6 Mutations
Array comparative genomic hybridization analysis excluded aneuploidy in each of the probands. To identify pathogenic coding or regulatory single nucleotide variants or insertion/deletions, WGS was performed on 21 individuals comprising the 5 families. Each sample yielded ≈1.5 billion 101 base paired end reads which passed quality control standards with 95% of the reads mapping to the genome. After marking and filtering out duplicate reads, >99% of the hg19 human reference genome had coverage. The average depth across the genome was 44X and an average of 95% of the gene body regions (exons, introns, and 5′ and 3′ untranslated regions) demonstrated a minimal read depth of 20 reads. As indicated in Figure 1, each proband had ≈5 million raw single nucleotide variant and insertion/deletion calls with an average Ti/Tv ratio of 1.98 and a heterozygosity rate of 1.01×10−3. On filtering for rarity, data quality, function and mode of inheritance, these calls were reduced to 50 coding and 33 enhancer or promoter variants among the 5 families, highlighting the power of family-based filtering. Gene-based filtering was then applied using 3 nonexclusive criteria: (1) candidate gene in at least 2 families; (2) known link to CHD; and (3) known link to DCM or heart failure. MYH6, encoding myosin binding protein 6, was the single gene that fulfilled >1 (all) of these criteria. MYH6 harbored compound heterozygous missense mutations in the probands in family 1H (exon 16: c.2111 T>A, p.I704N; exon 27: c.4136 C>T, p.T1379M) and 4H (exon 13: c.1763 A>C, p.D588A; exon 24: c.3619 G>A, p.E1207K; Figure 2A; Figure 1 in the Data Supplement). Each of the 4 mutations was also rare or unreported in >60000 unrelated individuals who comprise the Exome Aggregation Consortium (ExAC) database.22 None have been linked to human disease, 3 were predicted to be damaging by both Scale-invariant feature transform (SIFT)23 and polyphen-2,24 and each changed the physical property of amino acid residues. The chemical dissimilarity between 2 of the substitutions was predicted to be moderately radical by the Grantham matrix (Figure 2B). MYH6 has a residual variant intolerance score of 0.66%, indicating its tolerance of genetic variation is <99% of other genes.26 Moreover, the altered amino acid residues are highly conserved across species (Figure 3A).
Protein Modeling of MYH6 Substitutions Supports Their Biological Significance

The molecular architecture of myosin heavy chains is well established, enabling protein modeling to predict effects of mutations on structure and function (Figure 4A). Each pair of missense mutations identified in family 1H and 4H affect both head and tail domains (Figure 4B), with the following predicted consequences.

I704N

Although the role of I704 has not been characterized, MYH6 shares 80% amino acid identity with MYH2, encoding myosin heavy chain 2, allowing annotations to be transferred between the 2 proteins. Within MYH2, a critical hydrophobic residue triad was identified, which simulations found to be critical in the recovery stroke (Figure IIA in the Data Supplement). I704 is one of the structurally equivalent hydrophobic triad residues in MYH6 (Figure IIB in the Data Supplement). Accordingly, substitution of this hydrophobic isoleucine with a polar asparagine would be predicted to impede mechanical transduction of the recovery stroke leading to a less efficient contraction cycle.

D588A

Residue D588 is positioned behind a loop (residues 567–576), which has been computationally implicated and validated in actin binding (Figure III in the Data Supplement). We propose that D588 makes specific interactions with this loop, contributing to its conformational stability. Mutating this residue to alanine would remove these specific interactions leading to greater predicted conformational variability (Figure IV in the Data Supplement). We have performed unbiased molecular simulations which make a plausible case for the destabilization of the native binding-competent conformation and may have broad effects on the protein.

T1379M and E1207K

Sequence-based features were first assessed to determine whether the mutations observed would disrupt secondary structure. Consensus secondary structure predictions and their confidence scores across 40 residues flanking each mutation (20 on each side) revealed changes in the secondary structure class of 1 or 5 residues with the introduction of the E1207K and T1379M, respectively (Figure V in the Data Supplement). Analysis of the helical confidence in this region revealed that each mutation resulted in 6 residues with an altered probability of appearing in a helix, the majority having a higher propensity for the helical conformation. This provides a simple indication that these mutations may alter the local structure of the MYH6 coiled-coil domain (Figure V in the Data Supplement).

To identify a more precise effect on the helical structure, coiled-coil domain mutations were threaded onto a representative structural segment and dynamics simulated using crystallographic contacts to constrain sampling (Figure VI in the Data Supplement). Overall motion of residues flanking each mutation was diminished, complementing the secondary structure prediction of greater helical propensity. Moreover, by measuring the interhelical distance of each residue pair across the coiled coil unit, deviations from coiled-coil domain topology were predicted, with introduction of end-fraying, straightening of a bulge for T1379M and creation of a kink for
E1207K (Figure VII in the Data Supplement). Together, these computations indicate that the identified mutations could affect the local coiled-coil domain structure and thereby the dynamics or rigidity of the myosin filament.

Discussion

Critical Role for MYH6 in Cardiac Development and Function

MYH6, encoding myosin heavy chain 6, plays a vital role in myofibril assembly and proper heart development. MYH6 is a conventional myosin consisting of head, neck, and tail domains. The tail domains of 2 MYH6 proteins form a coiled coil that stabilizes the molecule so that the head domain is able to generate force through its interaction with actin. In both humans and rodents, MYH6/Myh6 is the minor ventricular myosin heavy chain isoform during fetal development. Consistent with the severe congenital heart defects we observed in patients with compound heterozygous missense mutations in MYH6, ablation of both copies of Myh6 in mice led to gross heart defects and in utero lethality between d11 and d12. Isoform switching at birth occurs in rodents, with...
Myh6 becoming the major adult isoform because of postnatal downregulation of Myh7. 32 Although MYH7 is the dominant cardiac myosin heavy chain isoform in humans, MYH6 expression in the heart persists from fetal life into adulthood, where it is actively engaged in force generation and muscle contraction. 32 Indeed, MYH6 has been shown to account for 30% and 7% of adult ventricular myosin heavy chain in humans at the mRNA and protein levels, respectively, and it is downregulated in human heart failure. 34 Mice heterozygous for Myh6 disruption had reduced levels of the transcript and protein, yet survived with no structural heart defects. However, they exhibited myopathic heart disease as evidenced by cardiac fibrosis, altered sarcomeric structure, and severe cardiac dysfunction characterized by reduced contractility and relaxation of the left ventricle. 33

Dominant MYH6 Mutations in CHD or Cardiomyopathy

Several human genetics studies have reported dominant heterozygous mutations in MYH6 linked to hypertrophic cardiomyopathy, 35,36 DCM, 36,37 or CHD primarily consisting of secundum atrial septal defects. 38–41 To our knowledge, there has been no previous report of cardiomyopathy in patients with CHD who harbor a heterozygous MYH6 mutation. Moreover, there has been no clear correlation between the MYH6 domain in which the mutation resides and cardiac phenotype. Heterozygous MYH6 mutations associated with cardiomyopathy trend toward later disease onset, 35,36 consistent with the latent systolic dysfunction observed in carriers of compound heterozygous MYH6 mutations. Dominant MYH6 mutations associated with CHDs have variable penetrance as highlighted by segregation studies of familial atrial septal defect, 38–40 which revealed several mutation carriers without structural heart disease. Variable intrafamilial expression was also demonstrated in a family where 4 MYH6 mutation carriers had a range of phenotypes including bicuspid aortic valve, coarctation of the aorta, ventricular septal defect, and subaortic stenosis. 41 Assessment of 24 heterozygous MYH6 mutations associated with cardiomyopathy or a CHD revealed that 88% are private mutations identified in a single proband 35–40 and 63% are reported in the ExAC database 22 with allele frequencies that range from 0.00001 to 0.001. Because no information on cardiac phenotype is provided in the ExAC database, it is unknown whether screening echocardiography would reveal occult cardiomyopathy in carriers of these rare MYH6 alleles.
Recessive MYH6 Mutations in HLH With Reduced Ejection Fraction

Using a family-based filtering strategy for WGS data, we demonstrate for the first time recessive MYH6 mutations in 2 individuals with HLH who also developed reduced systemic right ventricular function 3 and 11 years after Fontan palliation. Heterozygous mutations were clinically silent in family members, although they may be at risk for the development of DCM as they age. Although 2 of the 4 mutations had reported, albeit rare allele frequencies in the ExAC database (T1379M=0.00062; D588A=0.0021), this is not unexpected in disorders exhibiting autosomal recessive inheritance. Neither mutation is reported in the homozygous state and in silico predictive modeling supported the biological significance of both. Mutation is reported in the homozygous state and in silico predictive modeling supported the biological significance of both. In fact, these allele frequencies are not dissimilar from those of previously reported heterozygous cardiomyopathy or CHD-associated MYH6 mutations (0.00001–0.001). Even higher population-based allele frequencies have been identified in ExAC for heterozygous DCM-associated mutations in LDB3 (D117N; 0.0046) and CSRP3 (W4R, 0.0024), both of which were functionally validated.

Compound heterozygosity for MYH6 mutations may be sufficient for arrested left heart development in the 1H and 4H probands, yet environmental factors could be contributory. Although baseline expression levels of Myh6 during murine cardiogenesis do not show a discernible difference between the left and right ventricle, it is interesting to note that bisphenol A, an ubiquitous environmental chemical, has been shown to selectively downregulate MYH6 expression by ~42-fold in the left ventricle of the fetal heart. Similarly, the age-dependent development of right ventricular systolic dysfunction may be multifactorial, attributable to biallelic MYH6 mutations in the context of a vulnerable systemic morphological right ventricle exposed to volume and pressure overload, hypoxemia, and recurrent cardiopulmonary bypass. Of the 4 MYH6 mutations identified, the maternally inherited mutations were located in the head domain and predicted to impair power stroke recovery (I704N) or its interaction with actin (D588A). Both paternally inherited mutations (T1379M and E1207K) were located in the tail domain and predicted to affect the local structure of the coiled coil domain.

Study Limitations

A larger sample size and identification of additional mutations will be required to further establish genotype–phenotype relationships between MYH6, HLH, and latent ventricular systolic dysfunction. Future studies using patient-specific induced pluripotent stem cells could advance understanding of the effect of recessive MYH6 mutations on cardiomyocyte structure and function. For our study, successful completion of a Fontan circulation was a key inclusion criterion and subjects were aged 5 to 22 years at enrollment. Consequently, early poor outcome and mortality could account for under-representation of MYH6-associated ventricular dysfunction in our study cohort. Mutations in other unidentified genes may underlie adverse myocardial outcomes after Fontan operation. Although our filtering scheme identified 4 additional genes with links to CHD (RERE, MID1), DCM (TLR8), or heart failure (CACNB1), further investigation did not provide sufficient evidence to implicate them as a cause for disease in these families. The complex

Figure 4. Architecture of MYH6 and mutation modeling. A, A surface-rendered model of a single MYH6 peptide is shown in dark blue with the relative position of the 4 amino acid substitutions highlighted in red. An actin filament is shown in gray; both are modeled from Protein Data Bank coordinates using the sequence represented by UniProt ID P13533. A second MYH6 molecule is shown in semitransparent green to indicate the formation of the coiled-coil domain. Insets show the structures in greater detail. Within the MYH6 head domain, the relay helix and helix18 are shown in green and tan, respectively. B, Top, The cyclic interaction between the myosin head and actin filament. The insets highlight the proposed effects of each mutation as determined by molecular simulation.
phenotypic features associated with reduced levels of RERE in mice and Optiz G/BBB syndrome because of MID1 mutations in humans are not consistent with the isolated cardiac phenotype of the probands in families 5H and 3H. The toll-like receptor family is known to play a role in innate immunity and induction of cardiomyopathy, yet levels of TLR8 in the heart are minimal as compared with other TLR family members decreasing the likelihood that this isoform harbors a pathogenic mutation in the 1H family. Although CACNB1 is intriguing as a candidate gene for heart failure, it is difficult to predict the effect of the 2 promoter variants, each located in distinct regions with unique regulators. Moreover, mice lacking this subunit have severe skeletal muscle abnormalities, a phenotype not present in the proband of family 4H. Because of the relatively large number of inherited variants within these 5 families, final filters were applied that focused on genes linked to a cardiac phenotype, knowledge which remains incomplete. Our filtering strategy assumed monogenic bases of HLH and ventricular dysfunction. Although family-based filtering for variants that fit a specific inheritance model is a powerful strategy for identifying rare variants with major effect, this approach would not identify synergistic effects of mutations in >1 gene or cumulative impact of common variants with minor effects. Finally, although we performed comprehensive WGS, nonprotein coding regions, which comprise 98% of the genome, are more sparsely annotated. Interpreting the significance of mutations in these regions that might affect gene regulation is challenging.

Conclusions

In summary, we demonstrate that molecular genetic defects in MYH6, a protein vital for cardiac development and function, are associated with a phenotype characterized by left heart underdevelopment and impaired systemic right ventricular performance. These findings may have implications for predicting outcomes and developing new strategies to prevent heart failure in HLH.

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Disclosures

None.

References

Infants born with hypoplastic left heart, once a universally fatal congenital heart defect, are now surviving into adulthood owing to advances in staged, palliative surgical techniques. Unfortunately, the right ventricle may prove unsuitable as a durable systemic pump in individuals with a Fontan circulation, and eventual decline in myocardial performance may necessitate cardiac transplantation. In this study, whole genome sequencing in phenotypically characterized families led to discovery of recessive, compound heterozygous MYH6 mutations in 2 patients with hypoplastic left heart who developed heart failure 3 to 11 years after Fontan operation. Heterozygous MYH6 mutations have been independently identified in patients with other congenital heart defects and dilated cardiomyopathy. Uniquely, the current study links perturbation of this minor myosin heavy chain isoform to developmental arrest and latent myopathy of left and right ventricles, respectively. These findings uncover a heritable basis for heart failure in single ventricle circulations and may inform new strategies to predict and prevent adverse outcomes in patients with hypoplastic left heart.

CLINICAL PERSPECTIVE
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DATA SUPPLEMENT
METHODS

Whole genome sequencing and bioinformatics

Paired-end libraries were prepared using the TruSeq DNA v2 sample prep kit following the manufacturer’s protocol (Illumina, San Diego, CA). Each whole genome library was loaded into 4 lanes of a flow cell and 101 base pair paired-end sequencing was carried out on Illumina’s HiSeq 2000 platform using TruSeq SBS sequencing kit version 3 and HiSeq data collection version 1.4.8 software. Reads were aligned to the hg19 reference genome using Novoalign version 2.08 (http://novocraft.com) and duplicate reads were marked using Picard (http://picard.sourceforge.net). Local realignment of insertion/deletions and base quality score recalibration were then performed using the Genome Analysis Toolkit version 1.6-9 (GATK). Single nucleotide variants and insertion/deletions were called across all samples simultaneously using GATK’s Unified Genotyper with variant quality score recalibration.

In silico protein modeling

Visualization

Electrostatic surfaces were generated using APBS and visualized at an intensity of ±5 kT/e. Images were generated with PyMOL. Secondary structure prediction was performed using JPRED. Crystallographic coordinates are referred to by Protein Data Bank IDs and are available from www.rscb.org.

Structural Models

Initial models of conventional human myosin 6 (MYH6) were generated by homology modeling using Swiss-Model with coiled-coil segments specifically threaded onto the template 2FXM. The sequence represented by UniProt ID P13533 (version 152, July 9, 2014) was used in all myosin 6 models. Comparative analysis to conventional myosin heavy chain 2 (MYH2) used the
sequence represented by UniProt ID Q9UKX2. In modeling the coiled-coil domain, the assumption was made that any coiled-coil sequence of the proper length would be able to attain the crystal packing configuration seen in 2FXM. To restrain simulation dynamics closer to those attainable in a physiologic context, the closest 8 symmetry mates (copy of the molecule within the crystal lattice) were generated and held fixed in space while the dynamics of the central copy were sampled.

**Molecular Dynamics Simulations**

Molecular dynamics trajectories were computed using NAMD\(^9\) and analyzed using custom TCL scripts in VMD\(^10\) and the R programming language. The CHARMm22+CMAP force field\(^11\) was utilized with NAMD’s GBIS implicit solvation model. Structures were minimized for 5,000 time steps, heated to 300K over a period of 0.15 million time steps (MTS), and equilibrated for 0.25 to 1.0 MTS for coiled-coil domain (CCD) and head domain models, respectively. Following this setup phase, 0.5 to 1.0 MTS of production simulation was gathered. “Time steps” were chosen rather than a unit of time, since implicit solvent simulations speed-up the system’s kinetics. Hence, dynamics are sampled at a faster computational rate than explicit solvent simulations. In the case of the D588A mutation, the prediction of conformational variability was computed by molecular dynamics trajectories using Root-Mean-Square Deviation to the initial crystallographic conformation. Similarly, Root-Mean-Square Deviations were computed for the residues flanking the E1207K and T1379M to determine the impact of the mutation on CCD motion.
FIGURES AND FIGURE LEGENDS

Figure I. Cardiac phenotypic findings seen on computed tomography angiography of 1H (A & B) and 4H (C & D) patients. Multiplanar reformatting is performed to obtain typical 4-chamber views in A & C. Volume rendered 3D reconstruction at the aortic root is shown in B & D. (A) Dilated and dominant right ventricle with a hypoplastic left ventricle. Yellow arrow points to a muscular ventricular septal defect, creating functional subaortic stenosis. Red arrow points to an atretic mitral valve. (B) Mild (acquired) hypoplasia of native aortic root. Yellow arrowheads mark coronary artery origins. (C) Dilated and dominant right ventricle with no visible left ventricular cavity. Thickening of the posterior ventricular myocardium (white arrow) marks the severely hypoplastic left ventricle with cavity obliteration. (D) Hypoplasia of native aortic root due to aortic valve atresia. Yellow arrowheads mark the coronary artery origins. Ao, native aortic root; FC, Fontan conduit; LV, left ventricle; PA, native pulmonary artery root; RV, right ventricle.
Figure II. Comparative modeling of I704 indicates a critical role in recovery. (A) The MYH2 hydrophobic triad identified by Baumketner\textsuperscript{12} is shown for crystallographic coordinates 1W9L. (B) The structurally equivalent MYH6 hydrophobic triad involves I704 and is visualized from 4DB1.
Figure III. D588A likely controls the mobility of an actin binding loop. (A) MYH6 is shown in blue and actin in gray; the end of the relay helix is visible in green. Heavy atoms that are nearby and make up the binding interface are shown in ball-and-stick representation with charged nitrogens in blue, charged oxygen in red, and partially charged in light blue and pink. Probable salt bridges are represented by dotted yellow lines. The interface between MYH6 (B and C) and actin (D and E) is shown by electrostatic potential (B and D) with positive charge in blue and negative red, and residue hydrophobicity (C and E) with a higher degree of hydrophobicity in red. In each image, one molecule’s molecular surface is shown with closely interacting residues from the other overlaid in stick representation.
Figure IV. D588A leads to increased conformational entropy. (A) Root-mean-square deviations to the initial crystal structure across three equilibration replicates (thin lines) and one production simulation (thick lines) show the increased propensity to sample a wider conformational space for the mutant. The three replicates for D588 (blue) and D588A (orange) are shown (B) just after reaching 300K and (C) after a further 100,000 time steps (the final time step in A), emphasizing the greater conformational diversity for the mutant compared to wild type which remains close to the initial conformation. The production run shown was extended by a further one million time steps.
Figure V. Difference in secondary structure prediction for CCD mutations. The secondary structure prediction changes upon mutation for 5 and 1 residues for T1379M and E1207K, respectively, while the confidence that the residue is within a helix changes for 6 residues in both.
Figure VI. Diagram of molecular dynamics system used to evaluate coiled-coil domain mutations. (A) Crystal packing configuration realized in 2FXM and used in modeling coiled-coil dynamics. The central or mobile molecule is shown in blue with the 8 closest symmetry mates in black. The position in sequence of the mutation site is shown in red. Sixty-two residues were added on each side of the mutation in each sequence context and threaded onto this template. (B) Diagram of the coiled-coil domain showing alpha carbons as spheres and linked to their respective cross-helix partner by yellow lines. These were the distances monitored for calculating inter-helix distances.
Figure VII. Mutations are likely to alter local CCD conformation. Molecular dynamic trajectories are computed and compared using RMSF. (A,B) Each chain in the CCD is reported separately. Overall, the mutations suppress fluctuation, however, the conformation of the chain may be different. To identify local conformational differences, the inter-helix distance was plotted across the coiled-coil unit to determine sections that had moved to an alternate conformation during the simulation for (C) T1379M and (D) E1207K. Regions with notable differences between the wild type (WT) and mutant sequences have structural snapshots displayed along the abscissa.
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