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CLINICAL RESEARCH
Congenital heart disease

A rare missense mutation in *MYH6* associates with non-syndromic coarctation of the aorta

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Aims Coarctation of the aorta (CoA) accounts for 4–8% of congenital heart defects (CHDs) and confers substantial morbidity despite treatment. It is increasingly recognized as a highly heritable condition. The aim of the study was to search for sequence variants that affect the risk of CoA.

Methods and results We performed a genome-wide association study of CoA among Icelanders (120 cases and 355 166 controls) based on imputed variants identified through whole-genome sequencing. We found association with a rare (frequency = 0.34%) missense mutation p.Arg721Trp in *MYH6* (odds ratio = 44.2, $P = 5.0 \times 10^{-22}$), encoding the alpha-heavy chain subunit of cardiac myosin, an essential sarcomere protein. Approximately 20% of individuals with CoA in Iceland carry this mutation. We show that p.Arg721Trp also associates with other CHDs, in particular bicuspid aortic valve. We have previously reported broad effects of p.Arg721Trp on cardiac electrical function and strong association with sick sinus syndrome and atrial fibrillation.

Conclusion Through a population approach, we found that a rare missense mutation p.Arg721Trp in the sarcomere gene *MYH6* has a strong effect on the risk of CoA and explains a substantial fraction of the Icelanders with CoA. This is the first mutation associated with non-familial or sporadic form of CoA at a population level. The p.Arg721Trp in *MYH6* causes a cardiac syndrome with highly variable expressivity and emphasizes the importance of sarcomere integrity for cardiac development and function.

Keywords Coarctation of the aorta • Genetics • Sarcomere • *MYH6*

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Introduction

Coarctation of the aorta (CoA) is the most common birth defect of the aorta with an incidence of about one per 2500 live births.[1] It is defined by local narrowing of the proximal descending aorta and/or aortic arch, accompanied by bicuspid aortic valve (BAV) in more than 50% of cases and generally presenting as either neonatal heart failure or hypertension later in life.[2] Surgical or interventional treatment considerably improves outcome but risk of premature cardiovascular morbidity and mortality remains despite appropriate therapy.[3]

Coarctation of the aorta is primarily a non-familial or sporadic disease.[4] However, it has been shown to cosegregate in families with left-ventricular outflow tract obstruction (LVOTO) malformations, a mechanistically defined subgroup of congenital heart defects (CHDs) including CoA, BAV, congenital aortic stenosis, and hypoplastic left heart syndrome (HLHS).[5] As a group, the LVOTO malformations are markedly heritable (0.71–0.90) and have a high relative risk for first-degree relatives (36.9).[6] In addition, around 15% of individuals with CoA occur as part of a recognized genetic syndrome (e.g. 45, X, or Turner).[7]

Not much is known about genetic causes of non-syndromic CoA. Several studies have found mutations in families with LVOTO malformations and a few instances of sporadic CoA, both with and without concomitant CHDs. The most strongly implicated gene is *NOTCH1*,[8–10] encoding a transmembrane receptor that regulates cell fate during development. Mutations in other genes, including *MYH6*[11,12] *SMAD6*,[13] *NKX2-5*,[14] and *GATA5*,[15] have been found in one or few individuals with CoA. The *MYH6* mutations were found in two families, one with predisposition to atrial septal defect (ASD)[11] and the other to HLHS.[12] Some individuals in these families presented with CoA. In addition, knockout in mice of several genes found within copy number variants in individuals with CoA, including *MCTP2*,[16] *MATR3*,[17] and *FOXC1*,[18] have resulted in CoA-like phenotypes.

Methods

GWAS study design

Study samples

The CoA sample set included 120 Icelanders who received the discharge diagnosis of CoA at Landspitali, The National University Hospital (LUH) in Reykjavik, the only tertiary referral centre in Iceland, between 1984 and 2016. The individuals were diagnosed with CoA between the years 1950 and 2016, with most individuals (75%) diagnosed after 1990. The individuals diagnosed with CoA were identified either through diagnostic codes of CoA (ICD-9 code 747.1, ICD-10 code Q25.1) registered between 1990 and 2016 or procedure codes of CoA (WHO codes 1-273, 5-369, 5-382, and 5-387, NOMESCO codes FDJ 00, FDJ 10, FDJ 20, FDJ 30, FDJ 42, and FDJ 96) registered between 1984 and 2016. The diagnoses of CoA were confirmed and detailed phenotypic characteristics (Supplementary material online, Table S1) established through review of electronic and paper medical records at LUH. Coarctation of the aorta was defined as a non-syndromic congenital narrowing of the aorta, the diagnosis of which was confirmed by a cardiologist with echocardiography and/or cardiac catheterization. The individuals used as controls in the CoA GWAS analyses consisted of disease-free individuals randomly drawn from the Icelandic genealogical database and individuals from other genetic studies at deCODE.

In addition to CoA, we included in the study the following samples from the deCODE phenotype database: BAV, ASD, ventricular septal defect (VSD), patent ductus arteriosus (PDA), late onset aortic valve stenosis (AVS), sick sinus syndrome (SSS), atrial fibrillation (AF), heart failure (HF), ischaemic stroke (IS), hypertension (HTN), coronary artery disease (CAD), left atrial diameter (LAD), aortic root diameter (ARD), left ventricular end-diastolic diameter (LVEDD), electrocardiogram (ECG) data, thoracic aortic aneurysm, high-degree atrioventricular block, and hypertrophic cardiomyopathy (Supplementary material online, Supplementary methods).

All DNA samples used in the study are part of deCODE's biobank established in 1996 and built up since then through various genetic studies at deCODE.

The study was approved by the Icelandic Data Protection Authority and the National Bioethics Committee of Iceland. Study approval numbers were VSN-15-053, VSN-15-016, VSN-15-056, VSN-15-058, VSN-15-114, VSN-15-057, and 10-009-S1. Written informed consent was obtained from all study participants. The study complies with the declaration of Helsinki.

Genotyping, whole-genome sequencing, and imputation

For chip genotyping, 151 677 samples were typed with the Illumina HumanHap300, HumanCNV370, HumanHap610, HumanHap1M, HumanHap660, Omni-1, Omni 2.5, or Omni Express bead chips at deCODE. Long range phasing of all chip-genotyped individuals was performed with methods previously described[19,20] (Supplementary material online, Supplementary methods).

The whole genomes of 15 220 Icelanders were sequenced using Illumina technology to a median depth of 35X (Supplementary material online, Supplementary methods). The sequence variants identified in the 15 220 sequenced Icelanders were then imputed into 151 677 Icelanders who had been genotyped with various Illumina single nucleotide polymorphism chips and their genotypes phased using long-range phasing.[19,20] The imputation of the sequence variants, identified thorough whole-genome sequencing (WGS), into the chip typed long-range phased individuals was performed with the same model as used by IMPUTE.[21] The utilization of long-range phased haplotypes enables accurate imputation of variants with frequency down to approximately 0.02% in this data set. Using genealogic information on Icelanders from The Book of Icelanders,[22] the sequence variants were imputed into first and second-degree relatives of chip genotyped individuals (genealogical imputation),[23] to further increase the sample size for association analysis and to increase the power to detect associations. We identified 32.5 million high quality sequence variants (all with imputation information >0.8 that mapped to build hg38) that were tested for association with CoA under the multiplicative model.

Association analysis

In the association analysis were 120 individuals diagnosed with CoA and 355 116 individuals as controls, all with imputed genotypes. The sequence variants imputed were identified through WGS of 15 220 Icelanders ($n = 33$ individuals with CoA and $n = 15\,187$ individuals as controls). Of the individuals diagnosed with CoA, 39 were chip typed and long-range phased and of the individuals who were controls 140 661 were chip typed and long-range phased. These were imputed with the same model as used by IMPUTE. The remaining individuals ($n = 81$ CoA cases and $n = 214\,412$ controls), were not chip typed themselves but were first or second degree relatives of the chip typed individuals and imputed using genealogical imputation as described in Ref.[23]

To account for inflation in test statistics due to cryptic relatedness and stratification, we applied the method of linkage disequilibrium score

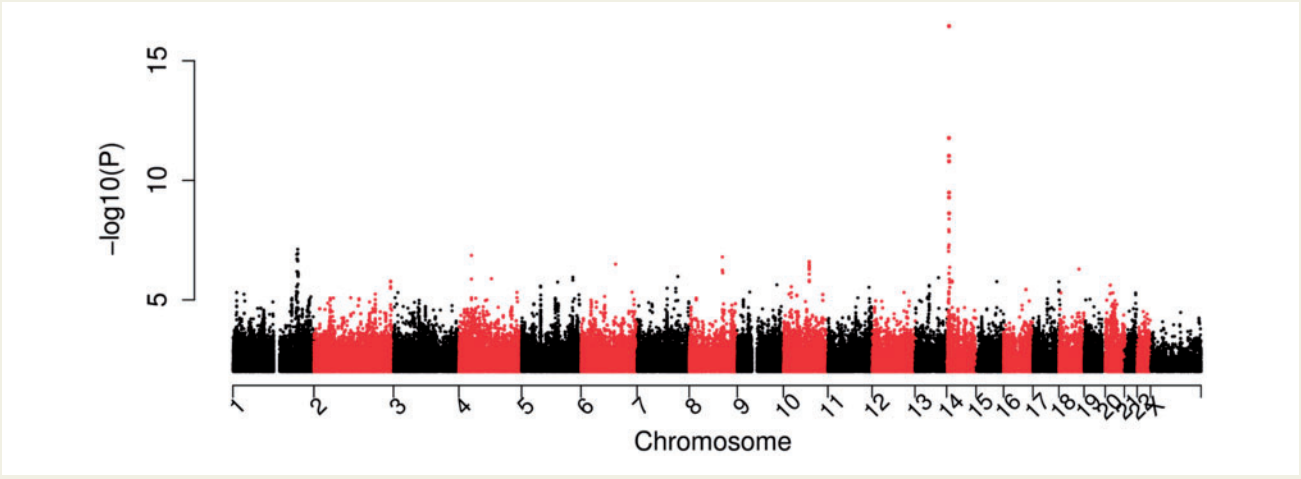

Figure 1 Manhattan plot of coarctation of the aorta genome-wide association study in Iceland. The *P* values ($-\log_{10}$) are plotted against their respective positions on each chromosome.

regression[24] (Supplementary material online, Supplementary methods). The estimated correction factor was 1.04 for the multiplicative model of the CoA association. To correct for multiple testing we used the weighted Holm–Bonferroni method[25] to allocate family wise error rate of 0.05 equally between four annotation-based classes of sequence variants (Supplementary material online, Supplementary methods). When testing the association of p.Arg721Trp with several other cardiac phenotypes, the individuals that served as controls consisted of disease-free individuals randomly drawn from the Icelandic genealogical database and individuals from other genetic studies at deCODE.

Phenotypic differences between carriers and non-carriers of p.Arg721Trp

To analyse if CoA carriers of the p.Arg721Trp mutation differed clinically from non-carrier individuals with CoA, we evaluated the frequencies of various clinical characteristics in these two groups with CoA (see Supplementary material online, Table S2). Fisher's exact test was used to test for significant difference in the mean frequency of the variants between non-carriers and carriers, and the odds ratio (OR) was calculated as [pa/(1-pa)]/[pc/(1-pc)], where pa and pc are the mean frequencies of the variants in non-carriers and carriers, respectively.

Results

A rare missense mutation in *MYH6* associates with coarctation of the aorta

To search for sequence variants that associate with non-syndromic CoA, we performed a GWAS including 120 Icelanders with CoA and 355 116 Icelanders who served as population controls. We observed a genome-wide significant association with CoA at chromosome 14q11 (*Figure 1*), explained by a rare (allele frequency = 0.34%) missense mutation p.Arg721Trp (c.2161C>T) in *MYH6*, encoding the alpha myosin heavy chain subunit (αMHC) in cardiac muscle. Alpha myosin heavy chain subunit is a main component of the sarcomere, the basic contractile unit of cardiac muscle.[26] P.Arg721Trp associates with CoA with an OR of 44.2 (95% confidence interval 20.5–95.5) and $P = 5.01 \times 10^{-22}$ (genome-wide significance threshold for missense variants was set at 6.5×10^{-8}, see Methods)[27] (*Figure 2*, *Table 1*). None of the genotyped individuals ($N = 151\ 677$) were homozygous for the mutation, consistent with its low frequency (1.8 homozygotes expected under Hardy–Weinberg equilibrium). Since we observed no homozygotes, we could not discriminate between the dominant and the multiplicative modes of inheritance.

The p.Arg721Trp mutation is located in exon 18 (out of 39 exons) of *MYH6* and leads to an arginine to tryptophan alteration at amino acid 721 (full-length protein 1939 amino acid) (see Supplementary material online, Figure S1). It is located in the converter domain of αMHC (see Supplementary material online, Figure S1 and S2), a small domain crucial in conveying a conformational change from the active site to the lever arm upon adenosine triphosphate (ATP) hydrolysis.[28] It is considered likely that the mutation alters protein function (SIFT = 0, PolyPhen = 0.99, MutationTaster = 0.93), probably by altering the folding of the converter domain.

There were 987 carriers of p.Arg721Trp among the 151 677 chip-typed Icelanders and eight of those (one per 123 carriers) were diagnosed with CoA. In line with low penetrance of the mutation for CoA, p.Arg721Trp carriers diagnosed with CoA did not cluster in families. However, 20% of the 39 chip-typed individuals with CoA carried p.Arg721Trp. Thus, while the penetrance of the mutation for CoA is low, it accounts for a large proportion of individuals with CoA in the Icelandic population.

The p.Arg721Trp mutation is not present in the Exome Variant Server, containing sequence data from 6503 individuals [Exome Variant Server, NHLBI Exome Sequencing Project (ESP), Seattle, WA, USA] (http://evs.gs.washington.edu/EVS/) (August 2016) and one copy was found in The Genome Aggregation Database (gnomAD), holding data from 126 216 exome sequences and 15 136 WGS unrelated individuals.[29] The p.Arg721Trp mutation thus appears to be absent from or present at a very low frequency in other populations.

We show the phenotypic characteristics of individuals with CoA in Supplementary material online, Table S1. About half were diagnosed

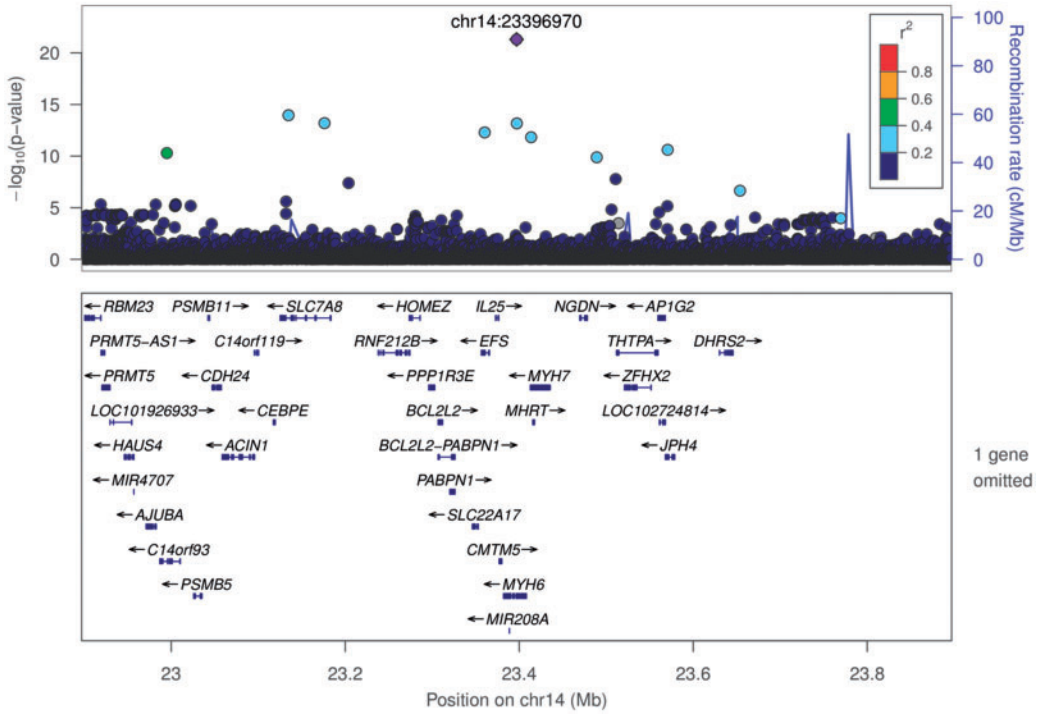

Figure 2 Region plot for the association of variants on 14q11 with coarctation of the aorta. Shown is a 1 Mb region on chromosome 14. The strongest association is with the missense variant p.Arg721Trp in *MYH6* located at position 23 396 970 on chromosome 14 (chr14: 23 396 970). The nine other variants shown are weakly correlated with p.Arg721Trp, r^2 between 0.6–0.4 (green) and 0.4–0.2 (light blue).

during the first month of life and three quarters during the first year of life. As expected,[3] CoA was more common in males than in females (1.6:1). About three quarters of individuals with CoA had other CHDs, most commonly BAV and VSD. Similar to other studies,[30] the aortic valve was bicuspid in about half of those with CoA.

To determine whether there are phenotypic differences between carriers ($n = 24$) and non-carriers ($n = 96$) of the p.Arg721Trp mutation within the CoA sample set, we evaluated the frequencies of various clinical characteristics in the two groups (see Supplementary material online, Table S2). Carriers were nominally more likely to present with mild rather than more critical and complex forms of CoA (OR = 4.2 and $P = 0.023$). We observed no other differences.

Association of p.Arg721Trp in *MYH6* with other cardiac diseases

We have previously demonstrated that p.Arg721Trp associates strongly with SSS and AF, atrial arrhythmias that are common in the elderly and frequently coexist.[31] With larger sample sizes, these associations have become stronger; however, previously reported association with thoracic aortic aneurysm is no longer significant (*Table 1*). In the context of assessing effects of AF risk variants on cardiac conduction, we have also recently shown that p.Arg721Trp associates with many ECG measures corresponding to a widespread effect on electrical function of the heart[32] (see Supplementary material online, Figure S3).

To further explore the effect of the p.Arg721Trp *MYH6* mutation, we tested it for association with additional cardiac phenotypes, including other CHDs, common heart diseases, and several echocardiogram variables (*Table 1*, Supplementary material online, Table S3 and Figure S3; significance threshold set at $P < 0.003$ (0.05/17 individual phenotypes tested). The p.Arg721Trp mutation associates with increased risk of several CHDs: BAV, VSD, ASD, and PDA (*Table 1*). As expected, the strongest association was with BAV (OR = 10.5 and $P = 7.3 \times 10^{-8}$). In addition, the mutation associates with late onset AVS. To assess if p.Arg721Trp associates with CHDs in the absence of diagnosed CoA, we re-tested for association after removing individuals with CoA from the analysis. Although the effect of p.Arg721Trp is consistently weaker, the associations remain (see Supplementary material online, Table S3). We cannot exclude the existence of undiagnosed CoA in these individuals. The mutation also associates with HF and IS and with LAD but not with other variables derived from the echocardiographic data such as ARD or LVEDD (*Table 1*). The p.Arg721Trp mutation did not associate with HTN or CAD.

Discussion

Through GWAS based on variants identified through WGS, we found a rare missense variant in the sarcomere gene *MYH6* that has a strong effect on the risk of CoA in the Icelandic population and explains a substantial fraction of CoA in Icelanders. The same mutation

Table 1 Association of p.Arg721Trp with congenital heart defects and various cardiac phenotypes

	N_{aff}	N_{contr}	OR/effect (95% CI)[a]	*P*-value
Congenital heart defects				
Coarctation of the aorta	120	355 116	44.2 (20.5 to 95.5)	5.0×10^{-22}
Bicuspid aortic valve	208	293 346	10.5 (2.6 to 38.0)	7.3×10^{-8}
Ventricular septal defect	715	357 641	4.4 (1.9 to 10.0)	3.7×10^{-4}
Patent ductus arteriosus	594	357 762	4.9 (2.1 to 11.6)	2.3×10^{-4}
Atrial septal defect	657	353 096	3.3 (1.5 to 7.1)	0.0026
Cardiac conditions				
Sick sinus syndrome	3310	346 082	8.7 (6.8 to 11.2)	6.2×10^{-64}
Atrial fibrillation	13 471	374 939	2.4 (1.9 to 3.0)	1.1×10^{-14}
Aortic valve stenosis	2457	349 342	2.7 (1.8 to 4.0)	1.8×10^{-6}
Heart failure	10 480	353 508	1.8 (1.4 to 2.3)	2.3×10^{-6}
Ischaemic stroke	8948	369 624	1.5 (1.1 to 2.0)	0.0029
High degree atrioventricular block	1303	361 919	2.1 (1.2 to 3.5)	0.0092
Coronary artery disease	37 782	318 845	1.2 (1.0 to 1.5)	0.056
Hypertrophic cardiomyopathy	163	239 293	0.0 (0.0 to 4.5)	0.15
Thoracic aortic aneurysm	353	302 458	1.8 (0.6 to 5.3)	0.31
Hypertension	54 974	324 803	1.1 (0.9 to 1.3)	0.44
Echocardiogram				
Left atrial diameter	19 380		0.3 (0.1 to 0.5)	2.6×10^{-4}
Aortic root diameter	19 506		-0.1 (-0.2 to 0.1)	0.41
LVEDD[b]	5701		0.0 (-0.3 to 0.3)	0.93

Shown are the number of affected individuals and control individuals used in the association analysis for each of the traits.
[a]Estimated odds ratio (OR) or the effect in standard deviation and the 95% confidence interval (CI) for the association with p.Arg721Trp.
[b]Left ventricular end-diastolic diameter.

also associates with other CHDs, in particular BAV. It has a widespread effect on cardiac electrical function and associates strongly with atrial arrhythmias, both SSS and AF. This is the first mutation shown to associate with non-familial or sporadic form of CoA at a population level. The p.Arg721Trp mutation appears to be absent from other populations or if present, at a very low frequency. The Icelandic population is a founder population in that a small number of ancestors account for a relatively large proportion of genetic diversity in the current population. Hence, sequence variants that are very rare in more outbred populations, like p.Arg721Trp, may thus be more frequent in Icelanders.[33]

Myosin is a major component of the sarcomere, the building block of the contractile system of cardiac muscle. Myosin is an ATPase cellular motor protein composed of two heavy chains and two pairs of light chains. The two heavy chains are αMHC and beta myosin heavy chain (βMHC) encoded by *MYH6* and *MYH7*, respectively. Both αMHC and βMHC are expressed throughout the heart during embryonic cardiogenesis and βMHC continues to do so in the adult heart whereas αMHC expression becomes restricted to the atrium.[34] Expression of *MYH6* has not been detected in the aorta.[35]

The pathogenesis of CoA is not well understood. One of the main models of CoA pathogenesis, the haemodynamic theory,[2,36] maintains that cardiac lesions resulting in decreased left ventricular outflow promote development of CoA by reducing blood flow through the Foetal aorta. The p.Arg721Trp mutation could predispose to CoA by reducing blood flow through the Foetal aorta because of diminished contraction of the developing heart. This hypothesis is supported by overexpression studies in rat cardiomyocytes showing that the p.Arg721Trp mutation impairs sarcomeric structure[37] and by our ECG data demonstrating widespread effect of p.Arg721Trp on cardiac electrical function, including in the ventricles. Our hypothesis is compatible with the fact that *MYH6* is expressed in the ventricles during the development of the heart but not in the aorta.

Very rare mutations in *MYH6*, other than p.Arg721Trp, have been linked to various CHDs,[11,12,38] particularly familial ASD[39,40] and both dilated and hypertrophic cardiomyopathy.[41] In all instances, these mutations have been restricted to a few sporadic cases or too few families. In two of these families, one with predisposition to ASD[11] and the other to HLHS,[12] some of the affected family members had other cardiac defects, including CoA. p.Arg721Trp in *MYH6* differs from these rare familial mutations in that it associates with CoA at the population level and explains about 20% of individuals with CoA in Iceland.

The main limitation of the study is the small size of the CoA sample set. A larger set might have facilitated detection of more variants associating with CoA with weaker effects than observed for p.Arg721Trp, and allowed a better estimate of the effect (OR), penetrance and the fraction of CoA cases explained by the mutation.

Conclusion

In conclusion, our findings give insights into the pathophysiology of CoA, supporting the haemodynamic theory of the pathogenesis.

Moreover, the pleiotropic effect of p.Arg721Trp in *MYH6* suggests it causes a cardiac syndrome with highly variable expressivity that is difficult to understand clinically without sequence information. Furthermore, these data emphasize the importance of sarcomere integrity for cardiac development and function.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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