Exome sequencing identifies a novel mutation in the MYH6 gene in a family with early-onset sinus node dysfunction, ventricular arrhythmias, and cardiac arrest

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Introduction

Cardiac arrhythmias leading to sudden death can be the first manifestation of an underlying genetic heart disease such as an inherited cardiomyopathy or primary arrhythmogenic disorder. In a number of cases, a clinical diagnosis cannot be established despite comprehensive clinical review. Genetic investigations can aid clinical diagnosis and uncover new disease loci and mechanisms related to cardiac arrhythmias and sudden death.

Sudden cardiac death can be the first manifestation of an underlying heart condition in otherwise healthy individuals. The predominant cause of sudden cardiac death in those older than 35 years is coronary artery disease. In those younger than 35 years, sudden cardiac death can be caused by a genetic heart disorder, which can be structural (eg, hypertrophic and dilated cardiomyopathies) or arrhythmogenic (eg, long QT syndrome [LQTS], catecholaminergic polymorphic ventricular tachycardia [CPVT], and Brugada syndrome).1 However, there remains a group of individuals in whom the cause of sudden cardiac death is unknown.2

Remarkable advances over the past 2 decades have helped discover genetic contributions to cardiac arrhythmias and sudden death. Defects in different cardiac ion channel genes have been linked to specific forms of inherited arrhythmogenic disorders.3 In cases where idiopathic ventricular fibrillation is the first sign of cardiac dysfunction and no cause is demonstrable on clinical evaluation, genetic testing has identified the underlying cardiac disease.4 Furthermore, a positive genetic result allows predictive testing of asymptomatic family members at risk of premature death. Nevertheless, some cases have no identifiable mutation in known disease-causing genes and therefore highlight the necessity to discover new susceptibility genes for inherited arrhythmogenic disorders.

Advances in next-generation sequencing platforms and targeted DNA capture strategies allow vastly greater capacity to sequence many thousands of genes at once, offering the ability to identify new genetic loci and mechanisms for cardiac electrical abnormalities.5 Herein, we describe the clinical and genetic investigation of a 3-generation family with a spectrum of cardiac arrhythmia phenotypes among 4 first-degree relatives, including sinus node dysfunction, ventricular fibrillation, and sudden cardiac arrest.

Methods

Clinical assessment

Clinical evaluation of family members was performed at the Genetic Heart Diseases Clinic, Royal Prince Alfred Hospital, Sydney, and the Heart Centre for Children, The Children’s Hospital at Westmead, Sydney, Australia. Clinical assessment included detailed family history, physical examination, 12-lead electrocardiogram (ECG), transthoracic echocardiography, 24-hour Holter monitoring, and cardiac magnetic resonance (CMR) imaging. All family members provided written informed consent, including parental consent for children.
GENETIC ANALYSIS

Commercial genetic testing of the index case for mutations in 6 common familial LQTS genes (KCNQ1, KCNH2, KCNE1, KCNE2, KCNJ2, and SCN5A) and the CPVT gene (RYR2) was previously performed, and no pathogenic mutations were identified in the index case. Therefore, whole exome sequencing and analysis of the proband was performed as described previously. In brief, the DNA sequencing library was prepared and enriched for the Illumina TruSeq Exome and paired-end sequenced on an Illumina HiSeq2000 system (Macrogen Facility, Seoul, Korea). Single nucleotide variants (SNVs) and insertions, deletions (Indels) causing a nonsynonymous, nonsense, altered splice site or a frameshift change and with a minor allele frequency (MAF) of <1% in the 1000 Genomes Project (http://www.1000genomes.org/) and 6500 control exomes from the NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVS/) were retained. Variant validation and cosegregation in family members were determined by Sanger sequencing.

All studies were carried out with approval of and in strict accordance with the Sydney Local Health District Ethics Review Committee.

RESULTS

CLINICAL FEATURE OF A FAMILY

We present a 3-generation Australian family with marked clinical heterogeneity. The clinical characteristics are summarized in Table 1, and the pedigree is illustrated in Figure 1A. The male index case (II:1) experienced an out-of-hospital sudden cardiac arrest at the age of 24 years. His initial cardiac rhythm was recorded as ventricular fibrillation. His initial postarrest ECG showed sinus rhythm with right bundle branch block and monomorphic ventricular ectopy. Echocardiogram and CMR imaging showed a mildly dilated left ventricle and mildly reduced ejection fraction, which subsequently improved. An electrophysiological study showed no inducible ventricular tachycardia. An implantable cardioverter-defibrillator (ICD) was subsequently implanted for secondary prevention, and he remained stable and free from appropriate device therapies after 4 years of follow-up.

Clinical screening of first-degree relatives was initiated. The father (I:1) and mother (I:2) of the index case had normal ECGs and exercise stress tests. His sister (II:3) had a clinical history of symptomatic palpitations and normal ECG, echocardiogram, CMR imaging, and 24-hour Holter monitoring.

All 3 children of the index case have been symptomatic. His eldest daughter (III:1) has sinus node dysfunction and has experienced episodes of syncope since the age of 9 months. A loop recorder documented junctional bradycardia with periods of asystole, with an episode of cardiac standstill as long as 13 seconds (Figure 1B, upper panel). She had a normal adrenaline provocation test, a normal corrected QT interval on a resting ECG, and a normal echocardiogram. She underwent permanent pacemaker (PPM) insertion at the age

| Table 1 | Clinical characteristics |
|---|---|
| ID | Current age (y) | Age at ECHO (y) | LVmax (mm) | PW (mm) | LVEDD (mm) | LA area (cm²) | ECG/Holter monitor | PPM | ICD | Genotype | Diagnosis |
| I:1 | 63 | 57 | 10 | 9 | 46 | 21 | Normal | N | N | --/-- | Normal |
| I:2 | 64 | 58 | 10 | 10 | 46 | 23 | Normal | N | N | +/-- | Normal |
| II:1 | 29 | 24 | 9 | 9 | 58 | 24 | Abnormal; RBBB; VEB | Y | Y | +/-- | IVF (RCA) |
| II:2 | 32 | 31 | 7 | 7 | 41 | 12 | Normal | N | N | --/-- | Normal |
| II:3 | 27 | 21 | 8 | 9 | 44 | NA | Normal | N | N | --/-- | Normal |
| III:1 | 7 | 7 | 4 | 4 | 2 | NA | 13 s of sinus arrest | Y | Y | +/-- | Sinus node dysfunction |
| III:2 | 5 | NA | NA | NA | NA | 28 | 11 s of sinus arrest | Y | Y | +/-- | Sinus node dysfunction |
| III:3 | 1 | 1 | NA | NA | 28 | NA | Partial RBBB | Y | Y | +/-- | Sinus node dysfunction |

+/-- = mutation positive; --/-- = mutation negative; ECG = electrocardiogram; ECHO = echocardiography; F = female; ICD = implantable cardioverter-defibrillator; IVF = idiopathic ventricular fibrillation; LA = left atrium; LVEDD = left ventricular end-diastolic diameter; LVmax = maximum left ventricular wall thickness; M = male; N = no; NA = not available; PPM = permanent pacemaker; PW = posterior wall thickness; RBBB = right bundle branch block; RCA = resuscitated cardiac arrest; VEB = ventricular ectopic beat; Y = yes.
of 3 years and has had no further events to date. Her PPM device was upgraded to an ICD at the age of 7 years.

The son (III:2) of the index case has also experienced episodes of syncope since the age of 12 months. Holter monitoring recorded an episode that correlated to 11 seconds of asystole with no ventricular escape (Figure 1B, lower panel). His ECG showed normal sinus rhythm with normal QRS axis and intervals. He was implanted with a PPM and has since continued to have episodes of brief unconsciousness, though less pronounced than previous episodes. His PPM device was upgraded to an ICD at the age of 5 years at the request of the parents. The youngest daughter (III:3) had several reported episodes of brief unconsciousness and unresponsiveness starting at the age of 3 months, with each episode lasting 5–10 seconds. Her ECG showed normal sinus rhythm with normal QRS axis and intervals. She was implanted with a PPM at the age of 11 months because of these recurrent syncopal events, the positive family history, and the request from the parents. No further episodes have been recorded to date.

**Genetic analysis and whole exome sequencing**

The index case underwent whole exome sequencing after commercial genetic testing of 6 LQTS genes (*KCNQ1, KCNH2, KCNE1, KCNE2, KCNJ2, and SCN5A*), and the CPVT gene (*RYR2*) failed to detect a pathogenic mutation. Targeted exome capture identified 208 heterozygous nonsynonymous, nonsense, or altered splice site SNVs after the aforementioned filtering steps. To identify a potentially...
causative variant, a Genomic Evolutionary Rate Profiling (GERP) score of ≥ 2, which is the general threshold to indicate evolutionary constrained nucleotides, was applied to missense variants to retain 149 SNVs (Online Supplemental Table S1). In addition, 15 rare InDels occurring in coding regions, or causing frameshifts or affecting the canonical splice site sequences, were detected (Online Supplemental Table S2). Variants in ion channel genes or genes associated with cardiac disease were selected for cosegregation analysis in the family (highlighted in Online Supplemental Table S1). Analysis revealed a rare heterozygous variant in the cardiac muscle alpha-myosin heavy chain 6 gene MYH6 (NM_002471.3: c.1960C>T; p. Arg654Trp). This variant has been identified in 1 of 61,425 exomes (MAF = 0.000008) of the Exome Aggregation Consortium data (http://exac.broadinstitute.org) and is absent from the 1000 Genomes Project and our internal exomes from 166 unrelated individuals with diverse cardiac pathologies. Genotyping family members confirmed cosegregation of the MYH6 Arg654Trp variant with disease in all symptomatic individuals (III:1, III:2, and III:3; Figure 1C). The variant was present in 1 clinically unaffected female (I:2; Figure 1C). The father (I:1) and sister (II:3) of the index case do not carry the variant.

Discussion
We present a 3-generation Australian family with both severe and diverse cardiac arrhythmia manifestations of disease in which a heterozygous mutation in the MYH6 gene (Arg654Trp) was identified by whole exome sequencing and demonstrated to cosegregate with all affected family members. The diverse clinical features observed in this family include early-onset sinus node dysfunction and a resuscitated cardiac arrest due to ventricular fibrillation, reflecting the pleiotropic nature of MYH6 mutations. In this study, we provide further support to directly link a mutation in MYH6 to heritable cardiac conduction abnormalities using segregation analysis.

To date, there has only been 1 report establishing the importance of MYH6 and the development of cardiac arrhythmias. Holm et al performed a genome-wide association study and whole genome sequencing in Icelandic individuals and observed a strong association between a rare missense variant c.2161C>T (p. Arg721Trp) in MYH6 and sick sinus syndrome cases. In addition, in combination with other common MYH6 variants, the c.2161C>T variant associates with decreased heart rate and prolongation of the PR interval in individuals who have not been diagnosed with sick sinus syndrome, indicating that variants in this sarcomere gene are directly linked to cardiac conduction in humans. The missense variant we identified at position c.1960C>T in MYH6 occurs in exon 16 and results in the Arg654Trp substitution of a highly conserved residue in the myosin globular head. The myosin head functions as the motor domain and is composed of actin-binding sites, the ATP-binding pocket, and the converter domain, which are all linked by flexible single-stranded joints and critical in conformational changes during muscle contraction. Relatively few mutations have been identified in the MYH6 gene, with those occurring in the myosin motor domain reported to cause a number of cardiac outcomes, including dilated and hypertrophic cardiomyopathies, as well as different forms of congenital heart defects.

The clinical features observed in our family are striking. Although the MYH6 Arg654Trp mutation cosegregated with disease in the family, we observed incomplete penetrance and variable expressivity. The first manifestation of disease was a resuscitated cardiac arrest due to ventricular fibrillation in the index case, while his 3 symptomatic children presented with sinus node dysfunction all within the first year of life. The presence of both ventricular arrhythmias and sinus node dysfunction in the same family is an unusual clinical finding. Interestingly, a mutation of the residue homologous to MYH6 Arg654Trp in the closely related cardiac myosin isoform MYH7 (NC_000014.8; p.Arg652Gly) results in hypertrophic cardiomyopathy. While there is currently no clear evidence of cardiomyopathy in our family, we cannot exclude the possibility that affected individuals will develop a structural anomaly in the future.

The MYH6 Arg654Trp mutation is predicted to be deleterious by software tools PolyPhen-2, SIFT, and MutationTaster. However, the exact mechanisms involved in the cardiac conduction abnormalities that we observe in this family remain to be determined. The expression of MYH6 is primarily limited to atrial tissues and is associated with faster rates of contraction. Cardiac functional studies in animal models with reduced MYH6 expression revealed defects in cardiac contraction, and loss of myh6 in zebrafish caused aberrant atrial contractility and triggered changes in ventricular morphology and function. Thus, MYH6 expression strongly affects contractile properties of both the atria and ventricles during development and disease pathology. Furthermore, recent investigation of the cardiac-specific microRNA, miR-208a, encoded within an intron of MYH6 has shown evidence of its importance as a modulator of electrical signals in the heart. Overall, the current available data suggest that MYH6 anomalies can cause cardiac conduction abnormalities. Given the limited pedigree size and enigmatic disease characteristics in this family, the role of MYH6 variants in the electrical system of the heart warrants further functional analyses.

Conclusion
We have identified the MYH6 Arg654Trp variant as a causative mutation in a family with dominantly inherited cardiac conduction disorders. Marked cardiac heterogeneity is observed, including arrhythmogenic abnormalities resulting in a sudden cardiac arrest event and symptoms of sinus node dysfunction. The effect of this mutation highlights the pivotal role of MYH6 in the maintenance of normal cardiac conduction and may provide insight into new mechanisms and pathways involved in disease pathogenesis.
Acknowledgment
We are thankful to the family members for their interest and participation in this study.

Appendix
Supplementary data
Supplementary material cited in this article is available online at http://dx.doi.org/10.1016/j.hrcr.2015.01.022.

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