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Digenic inheritance of mutations in the cardiac troponin (*TNNT2*) and cardiac beta myosin heavy chain (*MYH7*) as the cause of severe dilated cardiomyopathy

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ABSTRACT

Familial dilated cardiomyopathy (DCM) is characterized by ventricular dilation and depressed myocardial performance. It is a genetically heterogeneous disorder associated with mutations in over 60 genes. We carried out whole exome sequencing in combination with cardiomyopathy-related gene-filtering on two affected family members to identify the possible causative mutation in a consanguineous Iranian family with DCM.

Two novel variants in cardiomyopathy-related genes were identified: c.247 A > C; p.N83H in the Troponin T Type 2 gene (*TNNT2*) and c.2863G > A; p.D955N in the Myosin Heavy Polypeptide 7 gene (*MYH7*). Sanger sequencing and co-segregation analysis in the remaining family members supported the coexistence of these digenic mutations in affected members of the family. Carriers of either variant alone were asymptomatic.

In summary, we find that digenic inheritance of two novel variants in DCM related genes is associated with a severe form of DCM. Exome sequencing has been shown to be very useful in identifying pathogenic mutations in cardiomyopathy families, and this report emphasizes the importance of comprehensive screening of DCM related genes, even after the identification of a single disease-causing mutation.

1. Introduction

Dilated cardiomyopathy (DCM) is a rare heart disorder, characterized by cardiac dilatation and reduced systolic function. It has an estimated prevalence of 1:2500 in the general population. Familial DCM is responsible for up to 50% of the cases reported (Haas et al., 2015) and an autosomal dominant pattern of inheritance is seen in most familiar DCM pedigrees (Mestroni et al., 1999). Mutations in over 60 genes encoding for cytoskeletal, sarcomeric, and desmosomal proteins have been found to cause different forms of DCM (Perez-Serra et al., 2016). We identified a consanguineous family of Iranian descent with characterised DCM (Table 1) in which we sought to identify the underlying mutation using a whole-exome sequencing (WES) approach in combination with cardiomyopathy-related gene-filtering.

2. Patient data

We identified a consanguineous family of Iranian descent (Fig. 1 and Table 1) with characterised DCM (including severe left ventricular dysfunction, normal heart valve function, no clinical evidence of ischemic heart disease). The proband, a 31-year-old man (IV-5) was diagnosed at age 30 with DCM. Echocardiography showed a mildly enlarged left ventricle (LV), global hypokinesia and severe impairment of the LV global function (ejection fraction 20–25%). Echocardiography in his 37-year old sister (IV-6) also diagnosed with DCM (since the age of 34) (IV-7) identified moderate global hypokinesia, grade I diastolic dysfuntion and her left ventricular ejection fraction (LVEF) was also moderately reduced (35%). The size of her left ventricle was normal and she had mild

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Table 1
Clinical characteristics of family members. Abbreviations: F, female; IVS, interventricular septum; LVEF, left ventricular ejection fraction; LVIDd, left ventricular internal end-diastolic diameter; LVIDs, left ventricular internal end-systolic diameter; M, male; MR, mitral regurgitation; n/a, not available; NYHA, New York Heart Association; PW, posterior wall; LBBB, left bundle branch block; TR, tricuspid regurgitation.

Pedigree ID	Sex	Current Age (yrs)	NYHA class	LVEF (%)	LVIDd (mm)	LVIDs (mm)	IVS (mm)	PW (mm)	Valves	ECG
III-9	M	65	n/a	n/a						
III-10	F	60	II	15-20%	5.6 cm	4.3 cm	0.7 cm	0.6 cm	Moderate secondary MR is seen, Moderate TR	LBBB
IV-4	M	42	I	60%	4.8 cm	3.0 cm	0.8 cm	0.8 cm	Normal	Normal sinus rhythm
IV-5	M	31	II	20-25%	5.7 cm	5.2 cm	0.8 cm	0.9 cm	Normal	Normal Sinus Rhythm
IV-6	F	37	I I	35%	5.2 cm	4.2 cm	0.9 cm	1.0 cm	Mild MR is seen	Normal Sinus Rhythm
IV-7	M	50	I	n/a						
IV-8	F	36	I	60%	5.0 cm	3.1 cm	0.7 cm	0.7 cm	Normal	Normal Sinus Rhythm
V-1	M	22	n/a	n/a						
V-2	F	20	n/a	n/a						
V-3	F	5	n/a	n/a						

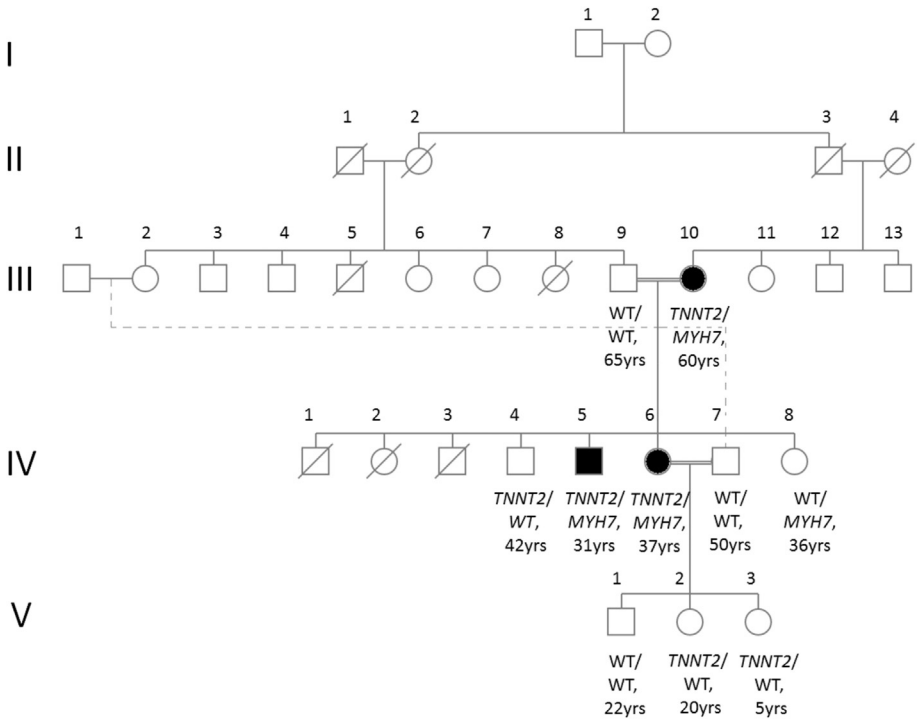

Fig. 1. Pedigree of family showing the age of individuals and segregation of the *TNNT2* and *MYH7* mutant alleles above and below respectively. WT=Wild-type alleles. Filled symbols indicate affected individuals. Diagonal lines across symbols indicate deceased individuals. IV-1, IV-2, IV-3 cause of death unknown, all <1 year of age.

mitral regurgitation (MR). Based on recent guidelines provided by the ESC working group on myocardial and pericardial diseases (Pinto et al., 2016), the phenotype in IV-5 and IV-6 can be more accurately defined as hypokinetic non-dilated cardiomyopathy as we did not observe significant dilatation and the left ventricular dysfunction could not be explained by abnormal loading conditions or coronary artery disease.

Their 60-year old mother (III-10) showed mildly enlarged left and right ventricles with severe systolic dysfunction, severe global hypokinesia, grade II diastolic dysfunction and severely reduced LVEF (15–20%). She underwent coronary artery bypass grafting (CABG) at the age of 52. Her mother had died at the age of 59 due to cardiac arrest, though the underlying cause is unknown and two of her siblings (III-11, III-12) underwent CABG at a similar age. III-11 a 60 y. o female was diagnosed with coronary artery disease at the age of 54. Echocardiography showed normal LV systolic function and size (LVEF:61%). RV and LA size were also normal and no other cardiac abnormalities were noted. III-12 a 56 y. o. male was diagnosed with coronary artery disease at the age of 50 and is hypertensive. Echocardiography showed good LV systolic function, LV size was normal, as was RV, LA and RA. No other cardiac abnormalities were noted. III-13 a 43 y. o. male showed normal systemic function by echocardiography at age 41, and is hypertensive. IV-1: died 28 days after birth (40 years ago), cause of death unknown and no additional medical data is available. IV-2: died 10 months after birth, cause of death unknown, no additional medical data available. IV-3: died shortly after birth, cause of death unknown, no additional medical data available. The two other living siblings of the proband (IV-4 and IV-8) appear to be healthy based on echocardiography and ECG, as do the three offspring of the affected sister (IV-6).

3. Methods

Genomic DNA samples from the two affected siblings (IV-5 and IV-7) were submitted for whole-exome sequencing (WES)

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following targeted exome sequence capture using the SureSelectXT Human All Exon v5 kit (Agilent). Sequencing was performed using the Illumina HiSeq2500 (Otogenetics Corporation, Atlanta, GA USA) to generate paired-end reads of 125 nucleotides with an average coverage of 30X. Reads were aligned to genome assembly GrCh38 with the Burrows-Wheeler Aligner (BWA,V.0.5.87.5). High quality indel and single nucleotide variant calling and annotation were performed using GATK v3.6 using standard filtering criteria (read depth $\geq$10%, genotype quality score $\geq$30).

Exome results were analysed for all modes of inheritance and variants initially filtered for potentially deleterious (as predicted by SIFT and MutationTaster) rare variants (Minor Allele Frequency, MAF <0.05) shared between the two siblings in all known DCM-associated genes (Supplementary Table 1). Three variants were predicted deleterious by both tools, and co-segregation analysis was carried out by Sanger sequencing in the available members of the family.

4. Results

Exome sequencing of the two affected siblings (IV-5 and IV-7) identified two potentially deleterious non-synonymous heterozygous variants in familial DCM-associated genes. The first missense variant, NM_000364.3 (*TNNT2*): c.247 A > C (p.(Asn83His)) occurs in the Troponin T Type 2 gene (*TNNT2*), and the second missense variant, NM_000257.3 (*MYH7*): c.2863G > A (p.(Asp955Asn)) occurs in the Myosin Heavy Polypeptide 7 gene (*MYH7*). Both variants are novel (absent from Exome Aggregation Consortium (ExAC) release ESP6500, as well as the Greater Middle East Variome Project database (http://igm.ucsd.edu/gme/) (Scott et al., 2016). We examined the putative functional effect of the *TNNT2* p.N83H mutation by using the prediction algorithms Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), SIFT (Sorting intolerant from tolerant) (http://sift.jcvi.org/www/SIFT_chr_coords_submit.html) and MutationTaster (http://www.mutationtaster.org) which predicted this amino acid change to be 'probably damaging' (HVAR (Human variation) score 0.984), 'deleterious' (SIFT score: 0), and 'disease causing' (Mutation Taster score 0.999) respectively. *TNNT2* is the tropomyosin-binding subunit of the troponin complex, regulating muscle contraction in response to alterations in intracellular calcium ion concentration. The Asn83His lies in the Tropomyosin (TPM1)-binding region of the gene. The *MYH7* p.D955N was also predicted damaging by the three different prediction algorithms examined (PolyPhen2 HVAR score 0.999, SIFT score:0, MutationTaster score 0.999). MYH7 (MHC-β) is the major protein comprising the thick filament in cardiac muscle and plays a substantial role in cardiac muscle contraction. The Asp955Asn mutation lies in the head—rod junction region (subfragment S2), a functionally important region for the interaction with the regulatory domain of myosin-binding protein C (MYBPC3) (Gruen and Gautel, 1999). We also identified a heterozygous mutation, NM_022114.3 (PRDM16): c.2452G > A (p.(Gly818Ser)), in a less well-established gene, *PRDM16*, encoding PR domain containing 16. This gene has been reported in one study to be associated with syndromic and non-syndromic left ventricular noncompaction cardiomyopathy (3 out of 75 individuals with 3 novel missense variants) and adult onset DCM (4 out of 131 individuals with 4 novel missense variants) (Arndt et al., 2013). PRDM16 has recently been suggested as a possible downstream target gene of the cardiac transcription factor TBX20 (Kodo et al., 2016). The variant identified in the family occurs at a frequency of 0.0044 in the ExAC population (all ethnicities). However, although there might be a role for PRDM16 in cardiac development, its role as a cardiomyopathy gene has been questioned (de Leeuw and Houge, 2014).

The PRDM16 variant did not co-segregate in the family (both parents (III-9 and III-10) were carriers, as was one of the unaffected offspring, IV-4), suggesting the variant is a polymorphism in this family. On the other hand from the co-segregation analysis it became apparent that the *TNNT2* and *MYH7* variants had been transmitted from the mother (III-10). The father (III-9) did not carry either of the variants. Three of the asymptomatic subjects (IV-4, V-2 and V-3) carried the *TNNT2* mutation alone, and one asymoptomatic subject (IV-8) carried the *MYH7* variant alone. Based on the available clinical information it appears that digenic inheritance of both variants is associated with the DCM phenotype, and carriers of either the *MYH7* or the *TNNT2* variant alone are asymptomatic.

5. Discussion

DCM is one of the leading causes of heart failure with high morbidity and mortality (Perez-Serra et al., 2016). Molecular genetic testing is crucial for selecting the correct therapy and management strategies for the disease, as well as to evaluate the prognosis of patients with inherited cardiomyopathy, and of their family members. The genetic diversity of cardiomyopathy has resulted in the necessity to routinely add new genes to existing clinical gene panels in order to improve the diagnostic yield. With decreasing costs, improved coverage, and comprehensive, hypothesis-free survey of ~20,000 genes, whole exome sequencing has become a valuable research tool for molecular diagnosis. We therefore employed WES in combination with cardiomyopathy-related gene-filtering to explore the possible causative mutation in this Iranian family with DCM.

A novel missense mutation (c.247 A > C p.N83H) in the thin filament Troponin T Type 2 gene (*TNNT2*) and a missense mutation (c.2863G > A p.D955N) in the thick filament Myosin Heavy Polypeptide 7 gene (*MYH7*) were identified. *MYH7* encodes the cardiac beta (β)-myosin heavy chain and the *TNNT2* gene encodes the thin filament contractile protein that links the troponin complex to tropomyosin in the sarcomere (Sweeney et al., 1998).

Although not common, digenic mutations have previously been described in a small number of cardiomyopathy cases, and often associated with increased clinical severity (Richard et al., 1999; Selvi Rani et al., 2015; Hoedemaekers et al., 2007; Ingles et al., 2005; Moller et al., 2009; Tsoutsman et al., 2008). However not all CM-related genes are sequenced following identification of an initial disease causing mutation. Therefore the estimates of double heterozygosity, may be inaccurate and potentially lead to inappropriate counselling and care of family members.

As part of a study of 105 unrelated subjects with DCM, Millat et al. reported a case of DCM due to digenic inheritance of mutations in both *MYH7* and *TNNT2*. The subject presented with a severe clinical phenotype with an age of onset <15 years (Millat et al., 2011). Our results support those findings as the three digenic carriers in the family are all severely affected. In addition, due to the availablility of extended family we observe that single mutation carriers (of either the *MYH7* or the *TNNT2* mutation alone) are unaffected, although there is the possibility that they may develop the disease later in life. Whilst the *MYH7* variant was not found in over 60,000 exomes in ExAC, nor in the GME Variome Browser, there is a report in the literature describing this variant on the same allele as a second missense mutation in *MYH7* (p.Asp545Asn/p.Asp955Asn) in a family with autosomal dominant non-compaction cardiomyopathy (Hoedemaekers et al., 2007) emphasising the clinical heterogeneity observed as a result of mutations in the same gene, and often within the same family.

In summary, we report a familial case of severe DCM resulting from digenic inheritance of two predicted damaging missense variants in the *TNNT2* and *MYH7* genes. Our study emphasizes the importance of comprehensive screening of DCM related genes,

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even after the identification of a single disease-causing mutation. Further work will be required to investigate whether digenic variants can adversely influence the clinical phenotype, including the age of onset, severity, and prognosis of the disease.

Ethics

The study conforms to the ethical guidelines of the 1975 Declaration of Helsinki. Ethical approval for the study was obtained from the Institutional Review Board (IRB) of Shahid Sadoughi University Of Medical Sciences, SSU, Yazd, Iran, Ethics Committee, Code: 254901. The family members were informed about the study and the informed consent was obtained from all participants.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmg.2017.06.008.

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