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Whole Exome Sequencing in a Large Pedigree with DCM Identifies a Novel Mutation in *RBM20*

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Abstract

Background—Familial dilated cardiomyopathy (DCM) is genetically heterogeneous and is associated with mutations in at least 40 different genes. Apart from *TTN* encoding the giant protein Titin, none of these genes have an expected diagnostic yield of more than 5 % complicating genetic diagnosis. Whole exome sequencing (WES) is a powerful alternative for the identification of the causal gene, however variant interpretation remains challenging. We report on WES in a large family with autosomal dominant DCM complicated by end stage heart failure and non-sustained ventricular arrhythmias in whom no causative mutation was identified using a targeted gene panel including 28 genes.

Methods and results—WES was applied on 2 affected cousins. Stringent filtering of the identified genetic variants was performed including population variant frequencies, in silico analysis, orthologous and paralogous conservation. Subsequently Sanger sequencing was performed for 10 potential disease causing variants in order to confirm the presence of the variant and to evaluate co-segregation. Only one variant in exon 9 of the RBM20 gene (c.2714T>A, p.Met950Lys, NM_001334363) showed full co-segregation in the 7 affected family members resulting in a maximum 2-point LOD score of 2.1 and suggesting this as the pathogenic mutation responsible for the phenotype. Recently mutations in RBM20 have been linked to arrhythmogenic dilated cardiomyopathy caused by defective splicing of the giant sarcomere protein titin and abnormal calcium handling.

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Disclosure of interests:

James R Lupski has stock ownership in 23andMe and Lasergen, is a paid consultant for Regeneron, and is a co-inventor on multiple United States and European patents related to molecular diagnostics for inherited neuropathies, eye diseases, and bacterial genomic fingerprinting. Other authors have no potential conflicts to report.

Conclusions—We report the identification of a novel mutation in RBM20 by WES in a large pedigree with DCM.

Keywords

RBM20; Whole Exome Sequencing; Dilated Cardiomyopathy

Introduction

Familial dilated cardiomyopathy (DCM) is a genetically heterogeneous disease associated with mutations in at least 40 different genes.(1) Apart from the giant protein Titin (*TTN*), none of these genes have an expected diagnostic yield of more than 5% complicating genetic diagnosis.(2) Genetic testing in clinical practice has evolved from gene by gene mutation scanning or Sanger sequencing to targeted gene panel testing. Whole exome sequencing (WES) is a powerful alternative for the identification of the causal gene in genetically heterogeneous diseases, however variant interpretation remains challenging. Identification of the causal gene in DCM helps in appropriately counseling the family, arrange proper follow-up for mutation positive family members and dismiss mutation negative family members from further follow-up. Some recent data even suggest gene or mutation specific risk stratification and treatment, for example in *LMNA* mutation carriers and in a Dutch cohort with a PLN founder mutation.(3, 4) We performed WES in a large Caucasian family with autosomal dominant DCM complicated by end stage heart failure and non-sustained ventricular arrhythmias. The index of this family was evaluated previously by means of targeted gene panel analysis including 28 genes, but no causal mutation was found.(5)

Methods

Sequencing

Whole exome sequencing was performed at the Human Genome sequencing center (HGSC) at Baylor College of Medicine through the Baylor-Hopkins Center for Mendelian Genomics initiative. Using 0.5 μg of DNA an Illumina paired-end pre-capture library was constructed according to the manufacturer's protocol with modifications as described in the BCM-HGSC protocol.(6) Six pre-captured libraries were pooled and then hybridized in solution to the HGSC VCRome 2.1 design(7) (42Mb NimbleGen, Cat. No. 06266380001) according to the manufacturer's protocol *NimbleGen SeqCap EZ Exome Library SR User's Guide* with minor revisions. The sequencing run was performed in paired-end mode using the Illumina HiSeq 2000 platform, with sequencing-by-synthesis reactions extended for 101 cycles from each end and an additional 7 cycles for the index read. With a sequencing yield of 5.1 Gb, the samples achieved 89% of the targeted exome bases covered to a depth of 20X or greater. Illumina sequence analysis was performed using the HGSC Mercury analysis pipeline which moves data through various analysis tools from the initial sequence generation on the instrument to annotated variant calls.(8, 9) Detailed methodology is available upon request. Informed consent was obtained from all family members and the study was approved by the local ethics committee.

Variant filtering

Two distant affected family members were chosen for whole exome sequencing. First, shared heterozygous variants (single nucleotide variants, small insertions and deletions) located inside the exon or at the exon/intron boundary (up to 3 nucleotides intronic) were selected. Synonymous variants were excluded, except if they were located at the exon/intron boundary. Variants with a minor allele frequency of >0.1% in publicly available exome databases (1000 Genomes(10), Exome Variant Server(11), ExAC(12)) were excluded. Furthermore, variants that were present in an in-house exome cohort (N = 80) performed at the same laboratory for other disease entities were also excluded since these probably represent local genetic variation or incorrect calling. The remaining variants were evaluated using a comprehensive scoring system specifically developed for variants in cardiomyopathy genes that includes different *in silico* analysis tools, orthologous and paralogous conservation and population frequencies.(13) Variants of unknown significance and (likely) pathogenic variants were further evaluated by Sanger sequencing for co-segregation analysis. Finally, the variants with matching co-segregation were also evaluated using the ACMG-AMP criteria.(14) Co-segregation in the ACMG-AMP criteria was assessed using the recommendations of Jarvik and Browning.(15)

Results

Clinical data

The family of interest concerns a 3 generation pedigree with dilated cardiomyopathy (figure 1 and table 1). They first came to our attention in 2001. The index of the family (III-2) is a male (°1987) who was initially admitted in another hospital with heart failure due to a dilated cardiomyopathy with a decreased ejection fraction. He was transferred to our center in cardiogenic shock, after an apical thrombus embolized to his right carotid artery and left coronary artery. The resulting left hemiplegia fortunately subsided within hours, but the occlusion of both his lateral branches caused a large area of persisting akinesia in the posterolateral part of his already diffusely weakened left ventricle. A temporary left ventricular assist device was inserted, but six days after the initial presentation an urgent heart transplantation had to be performed due to refractory cardiogenic shock. Histology of the explanted heart showed diffuse myocardial necrosis with a lymphocytic infiltrate without clear cause. An underlying myocarditis might have triggered the cardiomyopathy, however viral infection could not be proven.

His twin sister presented 8 months later with an episode of congestive heart failure and a severely depressed left ventricular ejection fraction. Inotropic therapy and IV diuretics were temporarily started with good clinical response. Standard heart failure treatment was started including bisoprolol, enalapril and spironolactone. Since this first episode, she remained asymptomatic with recovery of the cardiac function (LVEF ± 50%). Their father (II-1) was diagnosed with DCM during screening after the cardiac transplant of his son. He had an asymptomatic mildly reduced LVEF (50%) with moderate dilatation (LVEDD 60mm) at presentation. Treatment with bisoprolol and losartan was started. During follow-up mildly symptomatic episodes of non-sustained ventricular tachycardia (polymorphic, up to 30 beats, up to 180 BPM) were documented on 24 holter recordings, however without syncope. The

LVEF fluctuated between 50% and 25% during follow up. There was one episode of mild congestive heart failure necessitating the addition of hydrochlorothiazide to his drug regimen in 2006. One of the paternal uncles of the index (II-4) was diagnosed with DCM in 1999 in another center. At presentation he had a very severely reduced LVEF (10%) and was evaluated for cardiac transplant at that time. However his cardiac function partially recovered with standard heart failure therapy. His LVEF fluctuated between 30 and 45% and he was admitted several times for decompensated heart failure. Since 1999 episodes of non-sustained ventricular tachycardia have been documented, but without syncopal events. He received an ICD in 2007. Since implantation, antitachycardia pacing terminated 4 episodes of VT, no shock was delivered. His two sons have an asymptomatic borderline (III-5) and definite (III-6) DCM.

The grandmother (I.1) developed symptoms of heart failure shortly after she gave birth to her youngest son (II.4) and died many years later at age 72. Patient II-3 suffered a myocardial infarction at age 38 and died 7 years later. Unfortunately more information is lacking as he was treated at another hospital.

Filtering and interpretation of pathogenicity

WES was performed on 2 distant affected family members (III-2 and III-6 in the pedigree). Filtering of variants is illustrated in figure 2. After filtering, 19 variants were scored using the pre-specified protocol. Of these, 9 were classified as variants of unknown significance and 1 as likely pathogenic. These 10 variants were further evaluated with co-segregation analysis (table 2). Only the variant in *RBM20* was present in all affected or borderline affected patients and not present in the unaffected individuals. The variant was classified as likely pathogenic according to the ACMG-AMP criteria due to strong co-segregation, absence from controls (1000G, ESP and ExAC) and the fact that missense variants in this gene cause disease. The LOD score for this variant was 2.1, the maximum score obtainable in this pedigree. A variant in *CPN1* was present in all tested individuals apart from one who did not have DCM. *CPN1* encodes kininase, which is not linked to cardiac phenotypes or expressed in cardiac tissue and therefore presumably has no effect on the DCM observed in this pedigree.

Discussion

Comprehensive gene panel testing is the current cornerstone of clinical DNA testing for cardiogenetic diseases. Especially in DCM, which is genetically very heterogeneous, this approach makes clinical DNA testing feasible.(2) However, a definite mutation can only be identified in about 40% of the cases.(2) Whole exome sequencing is a powerful alternative, since it covers the whole protein coding sequence of the genome. Therefore, it might unravel a mutation, which is located in a region, that is not yet known to be associated with a specific disease and thus not included in a dedicated disease panel. Since WES results in an enormous amount of genetic variants, identifying the disease-causing mutation remains similar to looking for a needle in a haystack. Therefore, we applied WES on 2 distant affected family members, thereby reducing the number of variants of interest to only 3.8% of the initial number. After some additional filtering steps a limited number of variants

remained, and co-segregation analysis by Sanger sequencing of these variants became feasible. The *RBM20* variant was the only that fully co segregated with the affected family members. At the time the proband of the current pedigree underwent genetic testing, *RBM20* was not yet established as a definite DCM causing gene and was not included on the gene panel that was performed earlier.

Mutations in *RBM20* (RNA Binding Motif 20) have been first identified in patients from 2 families with familial dilated cardiomyopathy in 2009.(16) In the following years, several mutations have been described.(17, 18) Most of these were located in an arginine serine rich mutation hotspot region in exon 9. In 2012, a comprehensive evaluation of the *RBM20* gene in a cohort of 283 individuals with DCM revealed a mutation in *RBM20* in 2.8%.(19) In contrast to the first reports, these mutations were equally distributed across the gene. Functional studies of mutations in the *RBM20* gene showed the importance of the gene in *TTN* splicing.(20) Missense mutations in *RBM20* result in increased expression of a longer titin isoform by acting as a splicing repressor.(21) However, *RBM20* not only regulates *TTN* splicing, it is also involved in splicing of several other genes that have been implicated in DCM.(20) Furthermore, it was recently shown that mutations in *RBM20* also lead to aberrant splicing of the calcium homeostasis related genes *CAMK2D* and *RYR2* and subsequent activation of L type Calcium channels, intracellular calcium overload and increased sarcoplasmic reticulum calcium content.(22) This leads to more pro-arrhythmic spontaneous calcium releases from the sarcoplasmic reticulum. The combination of disturbed titin splicing and function with disturbed calcium handling explains the more severe phenotype of *RBM20* mutation carriers compared to *TTN* mutation carriers.(22) Indeed, a recent meta-analysis showed that *RBM20* mutation carriers were transplanted at a significant younger age compared to other gene related DCM.(23) Also in our pedigree, the index patient presented with a refractory cardiogenic shock necessitating urgent heart transplantation, however he suffered a second ischemic insult on top of his genetic cardiomyopathy.

DCM is a heterogeneous disease with variable expression of disease severity in patients with the same disease causing mutation.(24) This is also evident from this pedigree, where one patient suffered severe heart failure necessitating urgent heart transplantation. Reasons for this variable expression have been explored and include environmental triggers (eg arterial hypertension, toxic exposure, cardiac inflammation, viral infection) but also co-inheritance of disease modulating genetic variants.(25) In contrast to disease causing mutations, these modulating variants might be more frequently observed in healthy population. We cannot exclude that some of the identified rare variants in this pedigree play a modulating role in disease expression and severity. However to unravel these more complex inheritance patterns, large multicenter genotype-phenotype studies are deemed necessary.

Conclusion

Whole exome sequencing of two distant affected family members with DCM is a powerful tool to identify a disease-causing mutation. This approach simplifies variant filtering and interpretation. *RBM20* should be routinely evaluated in patients with familial DCM.

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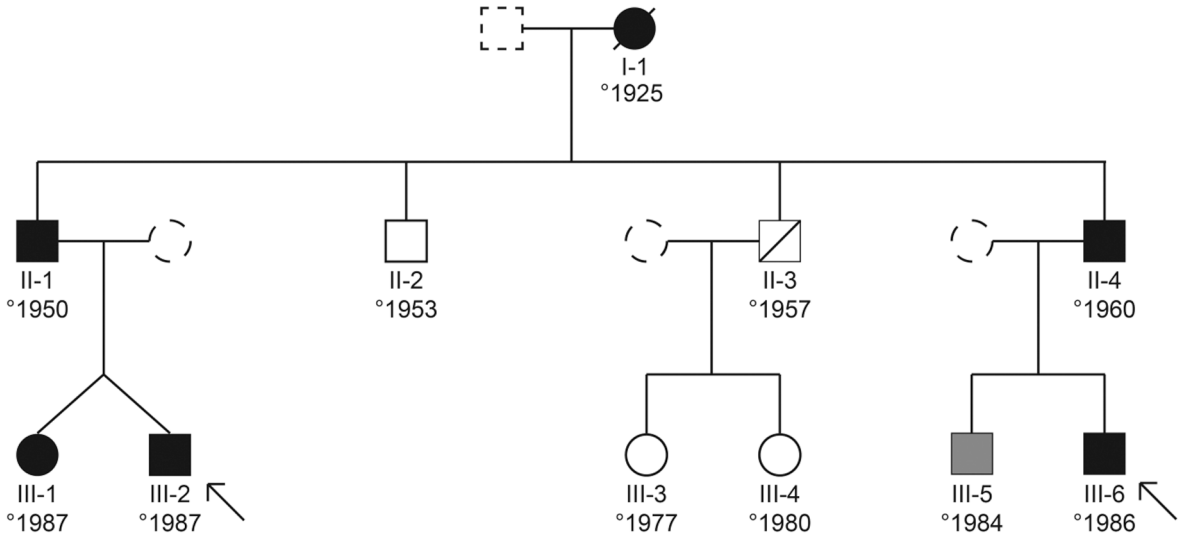

Figure 1: Pedigree.

Circles illustrate females, boxes illustrate males, filled symbols illustrate affected individuals, shaded symbols indicate borderline affected individuals, arrows indicate individuals that underwent whole exome sequencing

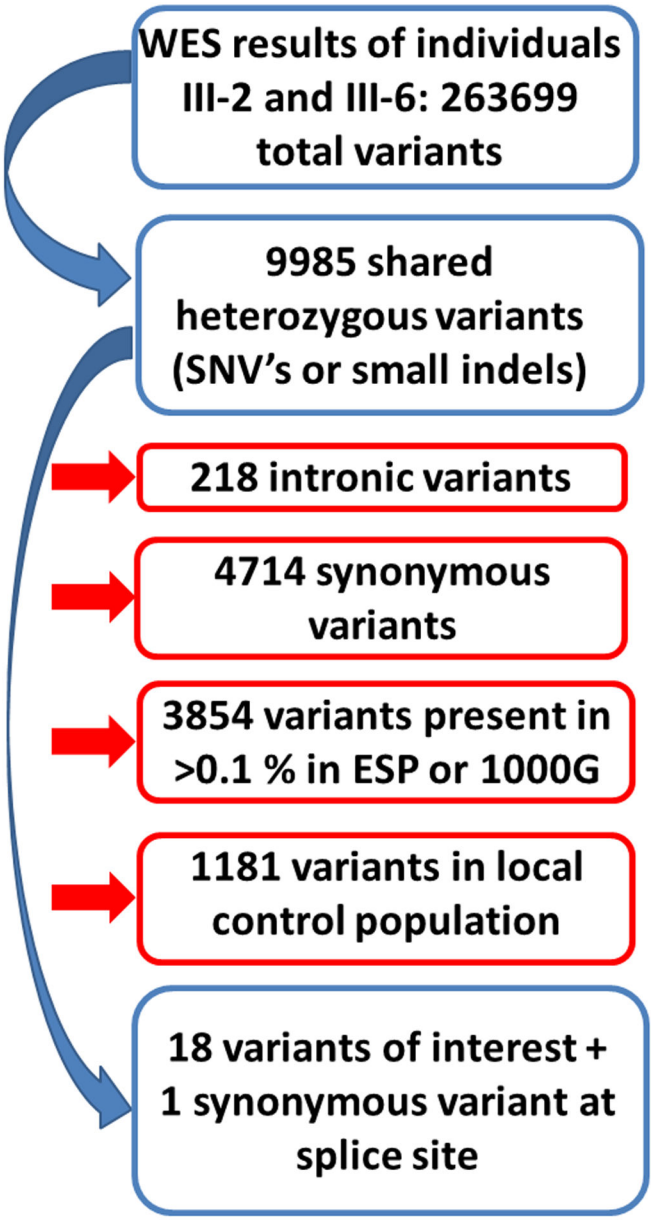

Figure 2: Filtering of whole exome sequencing data

Filtering steps applied to the variants that were discovered by whole exome sequencing. Red boxes indicate variants that were removed.

Table 1:

Clinical data

	Age	Phenotype	CHF	Treatment	ECG	LVEF (%)	LVEDD (mm)	nsVT	CAD	Alcohol
I-1	± 30	Peripartum CMP	+	NA	NA	NA	NA	NA	NA	NA
II-1	51	DCM	+	ARB + BB	LAHB	20	72	+	− (CAG)	−
II-2	48	neg	−	−	normal	71	52	−	NA	−
II-3	†45	ischemic CMP	NA	NA	NA	NA	NA	NA	+	NA
II-4	39	DCM	+	ICD + ACE-I + BB + digoxin	iLBBB	10	59	+	− (CAG)	NA
III-1	12	DCM	+	ARB + BB	normal	34	60	+	NA	−
III-2	13	DCM	+	urgent HTx	normal	25	65	−	+	−
III-3	24	neg	−	−	normal	60	45	−	NA	−
III-4	21	neg	−	−	normal	60	44	−	NA	−
III-5	17	borderline DCM	−	BB	normal	50	55	+	NA	−
III-6	15	DCM	−	BB	normal	40	56	−	− (CT)	+ (20U/w)

†= age death; ACE-I = Angiotensin converting enzyme inhibitor; Age = age at diagnosis or first evaluation in the case of a negative phenotype; ARB = Angiotensin receptor blocker; BB = beta-blocker; CAD = coronary artery disease; CHF = congestive heart failure; HTx = heart transplant; ICD = implantable cardioverter defibrillator; iLBBB = incomplete left bundle branch block; LAHB = left anterior hemiblock; LVEDD = left ventricular end diastolic diameter; LVEF = left ventricular ejection fraction; NA = not available; nsVT = non sustained ventricular tachycardia

Table 2:

Identified variants of unknown significance or likely pathogenic variants after filtering and applying the variant interpretation score system

								Phenotype									
								Positive						Borderline	Negative		
Gene	Peptide	Exon	C.	P.	Transcript	Variant score	ExAC	I-1	II-1	II-4	III-1	III-2	III-6	III-5	II-2	III-3	III-4
ALPPL2	Alkaline phosphatase	11	1385del	Gly462Alafs*16	NM_031313.2	Class 3 (59%)	35/105460	Not confirmed by Sanger sequencing									
CPN1	Kininase	6	939_940insA	Phe314Ilefs*14	NM_001308.2	Class 3 (64%)	0/121392	+	+	+	+	+	+	+	−	+	+
GNAT2	Cone specific transducin	6	698T>C	Val233Ala	NM_005272.3	Class 3 (46%)	1/121408	−	+	+	+	+	+	−	+	+	+
IRX2	Iroquois homeobox protein 2	3	1226G>A	Arg409Gln	NM_001134222.1	Class 3 (59%)	0/112390	+	+	+	−	+	+	+	+	−	−
KIAA0895	Unknown	5	1015C>T	Arg339*	NM_001100425.1	Class 3 (64%)	0/119518	−	+	+	+	+	+	−	+	+	−
NBN	Nibrin	2	93_94del	Ala32Hisfs*4	NM_002485.4	Class 3 (64%)	0/121378	+	+	+	−	+	+	−	+	−	+
RBM20	RNA binding motif protein 20	11	2714T>A	Met905Lys	NM_001134363.1	Class 4 (73%)	0/21696	+	+	+	+	+	+	+	−	−	−
ST3GAL4	Sialyltransferase 4	7	404G>A	Gly135Glu	NM_001254757.1	Class 3 (68%)	0/121184	−	+	+	−	+	−	−	−	−	−
TNS1	Tensin 1	13	671T>A	Ile224Asn	NM_022648.5	Class 3 (68%)	2/121390	−	+	+	+	+	+	−	+	−	−
ZNF135	Zin Finger protein 135	4	714C>A	Ser238Arg	NM_001164530.1	Class 3 (64%)	31/121358	−	+	+	−	+	−	+	−	−	−