Coding Sequence Mutations Identified in *MYH7*, *TNNT2*, *SCN5A*, *CSRP3*, *LBD3*, and *TCAP* from 313 Patients with Familial or Idiopathic Dilated Cardiomyopathy

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Abstract

Background: More than 20 genes have been reported to cause idiopathic and familial dilated cardiomyopathy (IDC/FDC), but the frequency of genetic causation remains poorly understood.

Methods and Results: Blood samples were collected and DNA prepared from 313 patients, 183 with FDC and 130 with IDC. Genomic DNA underwent bidirectional sequencing of six genes, and mutation carriers were followed up by evaluation of additional family members. We identified in 36 probands, 31 unique protein-altering variants (11.5% overall) that were not identified in 253 control subjects (506 chromosomes). These included 13 probands (4.2%) with 12 *β*-myosin heavy chain (*MYH7*) mutations, nine probands (2.9%) with six different cardiac troponin T (*TNNT2*) mutations, eight probands (2.6%) carrying seven different cardiac sodium channel (*SCN5A*) mutations, three probands (1.0%) with three titin-cap or telethonin (*TCAP*) mutations, three probands (1.0%) with two LIM domain binding 3 (*LDB3*) mutations, and one proband (0.3%) with a muscle LIM protein (*CSRP3*) mutation. Four nucleotide changes did not segregate with phentoype and/or did not alter a conserved amino acid and were therefore considered unlikely to be disease-causing. Mutations in 11 probands were assessed as likely disease-causing, and in 21 probands were considered possibly disease-causing. These 32 probands included 14 of the 130 with IDC (10.8%) and 18 of the 183 with FDC (9.8%)

Conclusions: Mutations of these six genes each account for a small fraction of the genetic cause of FDC/IDC. The frequency of possible or likely disease-causing mutations in these genes is similar for IDC and FDC.

Keywords: dilated cardiomyopathy, genetics

Introduction

In the past 10 years substantial progress has been made in identifying genes that cause dilated cardiomyopathy. Yet determining the mutation frequencies of these genes in patients with idiopathic or familial dilated cardiomyopathy (IDC or FDC) has been challenging for several reasons. Gathering large cohorts of carefully phenotyped FDC/IDC probands and their family members is time and effort intensive. In addition, locus heterogeneity (more than 20 genes have been described)[1,2] and allelic heterogeneity (mutations can involve any coding nucleotides) greatly complicate investigational strategies.[1] For these reasons there is a lack of comprehensive gene mutation reports of large FDC cohorts, a deficit that prevents the field from achieving a greater understanding of the genetics of dilated cardiomyopathy.

We utilized the resequencing service provided by the National Heart, Lung, and Blood Institute (NHLBI) to obtain mutational data from a large cohort of probands with FDC or IDC. Direct DNA sequencing is the gold standard in mutation detection; however, it involves considerable cost and effort. We therefore selected six of the more than 20 known FDC genes using a strategy designed to maximize the yield of genetic variants. The six genes included *β*-myosin heavy chain (*MYH7*), cardiac troponin T (*TNNT2*), the cardiac sodium channel (*SCN5A*), titin-cap or telethonin (*TCAP*), LIM binding domain 3 (*LBD3*), and muscle LIM protein (*CSRP3*). We present here the protein-altering variants identified in the disease cohort.

Methods

Patient population

Written, informed consent was obtained from all subjects, and the OHSU Institutional Review Board approved the project. The study included 313 probands (291 Caucasians, of whom seven were of Hispanic descent; 16 African-Americans, three Asians, and three Native Americans/Alaskan Natives), and used methods of clinical categorization of FDC versus IDC as previously described.[3,4] Families with confirmed and probable histories of familial disease[3] were classified as having FDC; those with histories consistent with possible FDC or a negative family history were classified as having IDC.[3] In cases classified as confirmed FDC, the patient and at least one closely related relative had IDC, defined as LVE accompanied by systolic dysfunction upon exclusion of other detectable causes of dilated cardiomyopathy (DCM).[3] A composite clinical description of 304 probands and their families and our FDC/IDC categories have previously been described[3] in detail; 302 of those 304 and an additional 11 IDC and FDC probands were included in this study.

Selection of genes for analysis

Using the published FDC literature available in early 2005, we estimated the number of exons that would need to be sequenced in order to find a single mutation in each gene, based upon the numbers of exons in that gene and an estimate of the frequency of that gene in dilated cardiomyopathy [(estimated frequency) × 1/number of exons)] (***Table 1***).[5–31] The estimated numbers of exons sequenced to find one mutation ranged from 150 (*LMNA*, the lamin A/C gene) to 6,333. A threshold of 1,000 exons was selected and included seven genes. This cohort was already undergoing sequencing for the *LMNA* gene[32]; therefore, six genes remained for analysis.

Genetic analysis

Genomic DNA was extracted from whole blood according to a standard salting out procedure.[33] Bidirectional sequencing was conducted for the following six genes: *MYH7*, *β*-myosin heavy chain, NM_000257, chr14:22951788-22974690; *TNNT2*, cardiac troponin T, NM_000364; chr1:198059798-198078462; *LBD3*/Cypher, LIM domain binding 3, AB014513, chr10:88418407-88484731; *TCAP*, titin cap, NM_003673; chr17:35075124-35076333; *SCN5A*, sodium

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Gene*	Protein	OMIM	Estimated mutation frequency	Number of Exons	Exons needed to sequence to find one mutation	References
LMNA	Lamin A/C	*150330	0.08	12	150	5–8
titin-cap TCAP	Titin-cap or telethonin	*604488	0.007	2	286	9
TNNT2	Cardiac troponin T	*191045	0.04	15	375	10–12
MYH7	β-myosin heavy chain	*160760	0.10	40	400	10, 13
LDB3	LIM-binding domain 3	*605906	0.03	13	433	14
SCN5A	Sodium channel	*600163	0.03	28	933	15, 16
CSRP3	Muscle LIM protein	*600824	0.005	5	1000	17
TNNC1	Cardiac troponin C	*191040	0.004	6	1500	18
TNNI3	Cardiac troponin I	*191044	0.005	8	1600	19
TPM1	α-tropomyosin	*191010	0.006	10	1667	20
VCL	Metavinculin	*193065	0.01	22	2200	21
ACTC	Cardiac actin	*102540	0.003	7	2333	22
ACTN2	α-actinin-2	*102573	0.009	21	2333	23
SGCD	δ-sarcoglycan	*601411	0.003	8	2667	24
DES	Desmin	*125660	0.003	9	3000	25
ABCC9	SUR2A	*601439	0.006	38	6333	26
MYBPC3	Myosin-binding protein C	*600958	?	35	?	13
TTN	Titin	*188840	?	363	?	27
MYH6	α-myosin heavy chain	*160710	?	39	?	28
EYA4	Eyes-absent 4	*603550	?	20	?	29
PLN	Phospholamban	*172405	?	2	?	30, 31

*These genes are autosomal. All mutations previously reported were autosomal dominant except for cardiac troponin I, which was recessive. This is an estimate based upon mutation frequency in FDC/IDC from published accounts, where available, and numbers of exons; see methods for details.

Table 1. Gene resequencing selection table.

channel, NM_198056, chr3:38564557-38666167; and *MLP*, muscle LIM protein, NM_001103, chr1:233175848-233253281. All exons and intron/exon boundaries were polymerase chain reaction (PCR) amplified by standard methods at SeattleSNPs under contract to the NHLBI resequencing service.

Samples from probands identified by the resequencing service as carriers of protein-altering variants, as well as any available samples from their relatives, were sequenced in our laboratory for confirmation and segregation analysis. Nucleotide changes were only evaluated if they were absent from all 253 (188 Caucasian, 24 African-American, 22 Asian, and 19 Hispanic) control samples analyzed at the resequencing center. Any possibly disease-causing nucleotide alterations identified in African-American samples were further evaluated in an additional 169 control African-American DNA samples in our laboratory, for a total of 193 African-American controls (386 chromosomes). Nucleotide changes were considered possibly disease-causing if they predicted a change in a conserved amino acid, a frameshift, premature truncation, or a missplicing event. Nucleotide changes were considered likely disease-causing if they met the above criteria and also segregated with disease in multiple affected individuals or were identified in multiple unrelated probands, or had previously been reported in association with dilated cardiomyopathy. Nucleotide changes, which obviously did not segregate with disease or were not conserved across species, were considered unlikely to be associated with IDC or FDC in the family in question.

Results

Bidirectional sequencing of six genes implicated in IDC/FDC was carried out on DNA specimens from 313 unrelated probands with IDC or FDC, 302 of whom have been previously described.[3] Protein-altering variants, none of which were present in 253 control specimens, were identified in 36 of 313 probands (11.5%) (***Table 2***).[10,11,18,34–43] Most were missense mutations, most altered highly conserved amino acids, and several had previously been reported in association with dilated cardiomyopathy or other cardiovascular disease (***Table 2***).

Of the six genes examined, the most commonly mutated was *MYH7*, with variants identified in 13 of the 313 probands (4.2%). All *MYH7* variants were considered possibly or likely disease-causing (***Table 2***). One mutation (Arg1500Trp), observed to segregate with disease (A.8), had been previously reported in a patient with IDC.[34] One proband (A.11) demonstrated a single mutant allele (Gly1808Ala) and no wild-type allele, indicating the subject was either homozygous or hemizygous for the mutation. Family history indicated no consanguinity, but parental DNAs were not available for analysis. Proband A.1 had both a possibly disease-causing novel *MYH7* variant, Arg237Trp and a possibly disease-causing *LDB3* mutation.

Gene/ Proband	Exon	Nucleotide change*	Amino acid change	Conser-vation†	Segre-gation¦	Disease-associated?	Diagnosis, FDC¶ or IDC‖	Previously reported cardiovascular disease	Reference
A. MYH7, β-myosin heavy chain									
A.1#	8	4231C>T	Arg237Trp	Yes		Possibly	FDC		
A.2	23	11900G>C	Val964Leu	Yes		Possibly	FDC		
A.3	23	11919C>T	Ala970Val	Yes		Possibly	FDC		
A.4	25	13547C>T	Arg1045Cys	Yes		Possibly	IDC		
A.5	26	14831G>T	Asp1096Tyr	Yes		Possibly	FDC		
A.6	26	14831G>T	Asp1096Tyr	Yes		Possibly	IDC		
A.7	30	17536C>T	Arg1359Cys	Yes		Possibly	IDC		
A.8	32	18666C>T	Arg1500Trp	Yes	Yes	Likely	FDC	DCM	34
A.9	34	19738G>A	Glu1619Lys	Yes		Possibly	FDC		
A.10	35	20125G>A	Val1691Met	Yes		Possibly	FDC	HCM	35
A.11‡	37	20709G>C**	Gly1808Ala	Yes		Likely	FDC		
A.12	39	21994C>G	His1901Gln	Yes		Possibly	FDC	Skeletal myopathy	36
A.13	38	21766G>A	Arg1863Gln	Yes		Possibly	FDC		
B. TNNT2, cardiac troponin T									
B.1	11/10	13712C>G	Arg134Gly	Yes	Yes	Likely	FDC		
B.2	11/10	13763C>T**	Arg151Cys	Yes		Likely	IDC		
B.3	12/11	14679G>A	Arg159Gln	Yes		Possibly	IDC		
B.4	13	16080C>T	Arg205Trp	Yes		Likely	IDC	DCM	18
B.5	13	16096_16098delAGA	Lys210del	N/A		Likely	FDC	DCM	10,11,18,37
B.6	13	16096_16098delAGA	Lys210del	N/A	Yes	Likely	FDC	DCM	10,11,18,37
B.7	13	16096_16098delAGA	Lys210del	N/A	Yes	Likely	FDC	DCM	10,11,18,37
B.8	13	16096_16098delAGA	Lys210del	N/A	Yes	Likely	FDC	DCM	10,11,18,37
B.9‡	14	16742G>T	Glu244Asp	Yes		Possibly	IDC	HCM	38
C. SCN5A, cardiac sodium channel									
C.1	6	36665C>T	Ser216Leu	Yes		Possibly	IDC	Long QT	39
C.2	6	36665C>T	Ser216Leu	Yes		Possibly	IDC	Long QT	
C.3	6	36683G>A	Arg222GLN	Yes	Yes	Likely	FDC		
C.4	12	46439G>A	Arg526His	Yes	No	Unlikely	IDC	Brugada Syndrome	40
C.5§	12	46577C>A	Ala572Asp	No	No	Unlikely	FDC	Long QT	41
C.6	13	51466C>T	Pro648Leu	Yes		Possibly	FDC	Long QT	42
C.7‡	28	99599T>C	Ile1835Thr	Yes	Yes	Likely	FDC		
C.8	28	100108C>G	Pro2005Ala	Yes		Possibly	IDC	Long QT	43
D. TCAP, titin-cap or telethonin									
D.1	1	1630G>A	Arg18Gln	Yes		Possibly	IDC		
D.2	2	1968G>A	Glu49Lys	Yes		Possibly	IDC		
D.3‖‖	2	2244C>G	Pro141Ala	Yes	No	Unlikely	IDC		
E. LDB3, limb domain-binding 3									
E.1	8	41461G>A	Ala371Thr	No		Unlikely	FDC		
E.2	12	57781G>A	Ala698Thr	Yes		Possibly	IDC		
E.3#	12	57781G>A	Ala698Thr	Yes		Possibly	FDC		
F. CSRP3, cysteine- and glycine-rich protein 3									
F.1	3	14108G>A	Gly72Arg	Yes		Possibly	IDC		

*Nucleotide numbering is per the SeattleSNPs resequencing service. Amino acid numbering is per previous publications. †Human sequence was compared to rat (r) and mouse (m); N/A, not applicable. ¶Probable FDC was considered FDC, and ‖possible FDC was considered IDC (see Methods). ¦Segregation means multiple affected carrying mutation and/or multiple nonaffected not carrying mutation; entry left blank because of insufficient clinical data and/or DNA specimens to assess segregation. #A.1 and E.3 is same proband with compound heterozygosity. **Homozygous mutation; Caucasian of Hispanic descent. ‡African-American. §Proband carries likely disease -causing presenilin 2 mutation. ‖‖Proband carries likely disease-causing lamin A/C mutation.

Table 2. Nonsynonymous nucleotide alterations in six FDC/IDC genes.

Six troponin T alterations were identified in nine probands (***Table 2***). In seven of the probands there was evidence to suggest that the variants were likely disease-causing. The variant in individual B.1 (Arg134Gly) segregated with the disease in other affected family members and was therefore considered likely disease-causing. IDC patient B.2, whose parents were second cousins, appeared to be homozygous for an Arg151Cys alteration. Individual B.4's Arg205Trp mutation had been previously reported in association with DCM, and was therefore considered likely causative.[18] A previously described disease-causing Lys210del[10,11,18] was observed in four unrelated probands (B.5, B.6, B.7, B.8). The remaining two *TNNT2* nucleotide alterations were considered possibly disease-causing. The Arg159Gln alteration in B.3 predicted the replacement of a conserved amino acid, as did the Glu244Asp variant in individual B.9, which was previously reported in a patient with hypertrophic cardiomyopathy.[38] However, in each of the latter two cases, there were not enough family members to establish segregation with disease.

Seven different protein-altering variants were identified in *SCN5A*, the cardiac sodium channel, among eight probands (***Table 2***). In six of the eight cases, the nucleotide changes were considered possibly or likely disease-causing. Mutations in *SCN5A* have been well established as a cause of the long QT syndrome Type III,[44] as well as of dilated cardiomyopathy.[15,16] Four *SCN5A* alterations (one of which was carried by two probands, C.1 and C.2) were previously reported in association with the long QT syndrome, and one, Arg526His, was previously associated with the Brugada syndrome (***Table 2***). All seven *SCN5A* variants changed conserved amino acids. Two novel variants (in individuals C.3 and C.7) segregated with FDC in their respective families and were considered likely disease-causing. However, the Arg526His alteration (C.4) reported in association with the Brugada syndrome did not segregate with DCM, and the Ala572Asp variant (in C.5) previously reported in association with the long QT syndrome did not alter a highly conserved amino acid nor did it segregate with DCM in the proband's large family. Therefore, the C.4 and C.5 variants were considered unlikely to be disease-causing in these families. No evidence of the Brugada or the long QT syndrome was present in the eight probands.

Three protein-altering variants were identified in *TCAP*, also known as titin-cap or telethonin, a part of the titin complex. Mutations in *TCAP* have been previously associated with DCM[9] and HCM.[9,45] One variant identified (D.3) did not segregate with disease, and this proband was also known to carry a likely disease-causing lamin A/C mutation (data not shown). The other two variants were considered possibly disease-causing.

Two nucleotide alterations were identified in *LDB3*, the LIM domain binding 3 gene, which encodes the ZASP (Z band alternatively spliced PDZ motif-containing) protein that functions in cytoskeletal assembly. Mutations in *LDB3* have previously been reported in association with IDC/FDC and left ventricular noncompaction.[14] One nucleotide change identified in an FDC proband (E.1) from whom no familial DNA specimens were available, occurred in an amino acid that was not well conserved. The identified mutation, Ala371Thr, predicted the replacement of the wild-type alanine with a threonine, which is the wild-type amino acid in the mouse. Thus, the Ala371Thr *LDB3* variant was considered an unlikely cause of the proband's FDC. The second *LDB3* protein-altering variant, Ala698Thr, was identified in two unrelated probands (E.2 and E.3), and was predicted to change a highly conserved amino acid. Notably, proband E.3 also carried an *MYH7* mutation in addition to the *LDB3* mutation, and therefore the relative contribution of the *LDB3* and *MYH7* mutations to the phenotype of proband E.3 could not be determined; for this reason the Ala698Thr mutation was considered possibly disease-associated. None of the *LDB3* mutation-carrying probands showed evidence of left ventricular noncompaction.

One protein-altering variant, Gly72Arg, was identified in *CSRP3*, a gene encoding cysteine rich protein 3 (also called cardiac LIM domain protein, or MLP), part of mechanical stretch sensing. This protein colocalizes with TCAP, a titin-associated protein.[17] The Gly72Arg variant predicted the replacement of a conserved amino acid and was therefore considered possibly disease-causing.

Eighteen of 183 FDC probands (9.8%) and 14 of 130 IDC probands (10.8%) carried possibly or likely disease-causing mutations. Because of the finding that possibly or likely disease-causing mutation frequencies in FDC and IDC probands were similar, we subdivided the FDC category into those with confirmed FDC and probable FDC, and subdivided the IDC category into those with IDC and those with possible FDC, according to our research database and as previously described.[3] The results were similar: for confirmed FDC there were 12 mutations in 134 probands (9.0%); for probable FDC there were six mutations in 49 probands (12.2%); for possible FDC, six mutations in 73 probands (8.2%); and IDC, eight mutations in 57 probands (14.0%).

Discussion

A longstanding issue in cardiovascular medicine has been the underlying cause of nonischemic dilated cardiomyopathy after all known and detectable causes have been excluded. This entity, known as idiopathic dilated cardiomyopathy (IDC), is at times present in two or more closely related family members; in such cases the assignment of familial dilated cardiomyopathy (FDC) has been made. It has recently been shown that genetic disease underlies some proportion of FDC,[1,2] but few studies have examined multiple genes in large cohorts to obtain preliminary frequency estimates.

We now present the frequency of possibly and likely disease-causing mutations in the coding regions and intron-exon boundaries of six genes implicated in FDC and IDC within a cohort of 313 probands with FDC or IDC. In DNA samples from these 313 probands we identified 36 subjects (11.5%) carrying a total of 31 unique protein-altering variants that were not observed in 253 control individuals. The genetic variants carried by 32 of the subjects (10.2%) were considered possibly or likely disease-causing mutations based upon their absence in control DNA samples, alteration of a conserved amino acid, segregation with disease, presence in two or more unrelated probands, and/or prior reports of the mutation in association with dilated cardiomyopathy.

Only a minority of the mutations showed segregation in families with multiply affected members or were previously published in association with FDC; therefore, most mutations were categorized as possibly disease-causing. We selected this conservative approach because in this multigene survey we were unable to provide functional data to substantiate the putative pathologic effects of these mutations. We note, however, that variations of the type here referred to as "possibly disease-causing" (i.e., rare nonsynonymous mutations not found in appropriately matched controls) would in some clinical genetic testing situations be considered likely causative.

We also observed similar mutation frequencies between IDC and FDC probands. Of the 183 probands with FDC, 18 carried likely or possibly disease-causing mutations (9.8%) similar to the 14 of 130 probands with IDC (10.8%). Similar results were observed by subdividing FDC and IDC into confirmed (9.0%), probable (12.2%), and possible (8.2%) FDC and IDC (14.0%) categories in accordance with our research database.[3] Although great care was taken in establishing and assigning probands

to these categories, these data suggest that the clinical and family history-based distinctions between IDC, possible FDC, probable FDC, and confirmed FDC may not be meaningful at the molecular level.

Another recent study from our group (which analyzed 324 probands, 304 of whom are in this study) identified 19 probands with likely disease-causing mutations in the coding region of *LMNA*, the gene encoding lamin A/C.[32] When these 19 probands carrying *LMNA* mutations are added to the 32 probands with possible or likely disease-causing mutations identified in this study, the overall frequency of putative genetic causation is 15.7% (51 of 324) within these seven FDC genes.

The 10.2% of IDC/FDC mutations identified in this study (or 15.7% including the lamin A/C data) likely underestimate the overall impact of genetic influence on IDC/FDC, as the coding regions of only a fraction of the 20+ known genes were examined. Further, it is likely that not all FDC-associated genes have been discovered. It is also possible that additional disease-associated genetic variation resides in regulatory areas (promoters, 5' and 3' untranslated regions) or introns of these genes, which would not have been detected in this study.

So while it can be argued that these studies have only scratched the surface toward discovering the full impact of gene variation on dilated cardiomyopathy, this preliminary evidence of the similar mutation frequencies for FDC and IDC (9.8% and 10.8%, respectively) for these genes suggests that the frequency of genetic causation in IDC, usually considered to be a sporadic, nongenetic disease, may in fact be similar to that of FDC. The similar frequencies of mutations observed in IDC and FDC, if substantiated in larger cohorts with more genes, suggest that the likelihood of genetic causation may be comparable with either an IDC or FDC diagnosis. Similar frequencies of genetic causation for familial and sporadic hypertrophic cardiomyopathy (HCM)[35] have been observed, and HCM is now principally considered a genetic disease.[46] Of the 197 HCM probands in one study, 172 had familial disease. The overall frequency of genetic disease was 63% in the familial cases and 60% in the sporadic cases.[35] By analogy, it is possible that a similar paradigm may emerge, in which IDC and FDC will be different points on a single spectrum of genetic disease. Resolution of these issues will require evaluation of these and other genes in larger cohorts of patients.

Limitations

This study is limited by the family data available for each proband. Large multigenerational pedigrees with multiple affected and unaffected members would improve the evidence of mutation segregation. Some probands have very small families, or have few or no surviving relatives available. However, because of this limitation we categorized mutations conservatively, assigning them only as "possibly" disease-associated. Linking each mutation to some adverse physiological effect in heterologous systems would also strengthen the evidence that these mutations may be disease-causing. However, in each of these genes nonsynonymous mutations changing conserved amino acids have been associated with DCM in prior studies (see ***Table 1*** and ***Refs. 1 and 2***), which supports our overall suggestion that these variants are possibly or likely disease-causing. The genes selected for this study were chosen for maximum utilization of the NHLBI resequencing resources, and were based on relatively limited frequency data available for some but not all FDC disease genes in early 2005. Hence, the study of additional FDC genes is merited.

Conclusion

The nonsynonymous mutations identified in these six genes implicated in FDC and IDC likely account for only a small fraction of the underlying genetic cause. The frequency of possibly and likely disease-causing nonsynonymous mutations in these genes is similar for IDC and FDC. Additional, larger studies are needed to confirm and extend these findings.

Acknowledgments

We thank the many families and referring physicians for their participation in the OHSU Familial Dilated Cardiomyopathy Research Program, without whom these studies would not have been possible. This work was supported by NIH awards RO1-HL58626 (Dr Hershberger) and 5 M01 RR000334. Resequencing services were provided by the University of Washington, Department of Genome Sciences, under U.S. Federal Government contract number N01-HV-48194 from the National Heart, Lung, and Blood Institute.

Conflict of Interest Disclosure: No conflicts.

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