Sarcomere mutations in cardiomyopathy with left ventricular hypertrabeculation

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Abbreviations: DCM, HCM, LVNC,

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Abstract:

Background: Mutations in the genes encoding sarcomere proteins have been associated with both hypertrophic and dilated cardiomyopathy. Recently, mutations in myosin heavy chain (MYH7), cardiac actin (ACTC) and troponin T (TNNT2) were associated with left ventricular noncompaction, a form of cardiomyopathy characterized with hypertrabeculation that may also include reduced function of the left ventricle.

Methods and Results: We employed clinically-available genetic testing on three cases referred for evaluation of left ventricular dysfunction and noncompaction of the left ventricle and found that all three individuals carried sarcomere mutations. The first patient presented with neonatal heart failure and was referred for left ventricular noncompaction cardiomyopathy. Genetic testing found two different mutations in MYBPC3 in trans. The first mutation, 3776 del a, Q1259fs, rendered a frame shift at 1259 of cardiac myosin binding protein C and the second mutation was L1200P. The frameshift mutation was also found in this mother who displayed mild echocardiographic features of cardiomyopathy with only subtle increase in trabeculation and an absence of hypertrophy. A second pediatric patient presented with heart failure and was found to carry a de novo MYH7 R369Q mutation. The third case was an adult patient with dilated cardiomyopathy referred for ventricular hypertrabeculation. He had a family history of congestive heart failure, including pediatric onset cardiomyopathy where three individuals in the family were found to have the MYH7 mutation R1250W.

Conclusions: Genetic testing should be considered for cardiomyopathy with hypertrabeculation.

Keywords: gene mutation, myosin heavy chain, myosin binding protein C, sarcomere, cardiomyopathy
Introduction

Mutations in the genes encoding sarcomere proteins are well established to cause hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM). The most common genetic causes of HCM are mutations in either \textit{MYH7} encoding \(\beta\) myosin heavy chain or \textit{MYBPC3} encoding cardiac myosin binding protein C. Together, these two genes account for 70-80\% of the mutational spectrum in HCM. In vivo, HCM is morphologically classified by thickening of the left ventricle (LV) that preferentially affects the septum but may also affect the left ventricular free wall. Apical variants of HCM, where the apex is preferentially targeted, have also been associated with sarcomeric gene mutations; the mutations associated with apical HCM display the similar genetic spectrum found in HCM and in some cases, apical variants have the identical gene mutations.

Noncompaction of the ventricular myocardium is characterized by a spongy morphological appearance of the myocardium that occurs in the LV and is most evident in the apical portion of the heart. During cardiac development, the embryonic myocardium forms as a hypertrabeculated ventricle thought to enhance blood flow to the developing but avascular myocardium.\(^1\) During embryonic weeks 5 and 8, the ventricular myocardium transforms from a hypertrabeculated morphology to a compacted layer, and this process is concomitant with coronary artery development. Myocardial remodeling begins at the base of the heart and over time progresses to the apex; the intertrabecular spaces form capillaries and the larger residual spaces flatten to become normal compacted myocardium. Left ventricular noncompaction (LVNC) is believed to arise from premature cessation of embryonic endomyocardial morphogenesis resulting in the presence of persistent trabeculated myocardium in the region where compaction arrested. Because of the base to apex progression, early termination of myocardial remodeling leaves the apex preferentially affected. Other portions of the ventricle including mid-ventricular lateral wall mid-ventricular inferior wall, and mid-posterior wall may also be involved.\(^2-4\)
LVNC may occur in the setting of other developmental defects such as cognitive impairment and developmental delay where it is referred to as "syndromic LVNC". Nonsyndromic LVNC refers to the absence of any extracardiac defects, and nonsyndromic LVNC may be familial. Nonsyndromic LVNC can be familial and may or may not be associated with structural cardiac malformations such as septal defects. LVNC can be difficult to diagnose because some degree of trabeculation may be normally present in heart. With the enhanced sensitivity of echocardiographic imaging, there has been an increased recognition of hypertrabeculation and LVNC. The American Heart Association reclassified LVNC as a primary cardiomyopathy in 2006. The clinical symptoms of LVNC range from none to severe heart failure. Like other forms of cardiomyopathy, LVNC may be associated with arrhythmias including ventricular tachycardia and atrial fibrillation. ECGs in adult and pediatric LVNC patients are typically abnormal with left or right bundle branch block, tachyarrhythmias and ventricular pre-excitation. Thromboembolic events are increased, and may arise from dislodged thrombi from the trabeculations of the LV and/or the associated arrhythmias.

The genetic etiology of LVNC includes both autosomal dominant and X-linked recessive inheritance. X-linked recessive inheritance of neonatal LVNC has been described with mutations in the G4.5 gene (TAZ). A single mutation in DTNA encoding α-dystrobrevin was associated with LVNC and congenital heart disease including ventricular septal defects, patent ductus arteriosus and hypoplastic left heart syndrome. LVNC has also been attributed to mutations in the gene encoding Cypher/ZASP. Overall however, mutations in DTNA, G4.5 and the LDB3 gene encoding Cypher/ZASP are rare causes of LVNC. Most recently, sarcomeric protein genes have been associated with isolated LVNC. Specifically, mutations in beta myosin heavy chain (MYH7), α-cardiac actin (ACTC), and cardiac troponin T (TNNT2) have been described. Where families and probands have been studied, sarcomere gene mutations have been identified in up to 17% of isolated LVNC cases. We now utilized clinical
genetic testing for sarcomeric gene mutations in three patients who were referred for having LVNC and hypertrabeculation in the setting of cardiomyopathy. Two patients were pediatric and one was an adult, and mutations were identified in all three. These data support the utility of using genetic testing to define the etiology and risks to relatives in LVNC.

Methods

Diagnostic criteria: The criteria of Jenni et al. were utilized to diagnose LVNC consisting of: 1) a two layered left ventricular wall structure with a compacted thin epicardial band (C) and the noncompacted endocardial layer (NC) of trabecular meshwork with deep endomyocardial spaces, with a maximal end systolic ratio of noncompacted to compacted layers (NC/C) greater than two; 2) the segments of noncompacted myocardium mainly involve the apex and the inferior mid and lateral mid of the left ventricular wall; 3) the visualization of blood flow from the ventricular cavity into the intertrabecular recesses by color Doppler imaging, 4) absence of coexisting cardiac abnormalities.

Molecular Analysis.

Genetic testing. Peripheral blood was collected from index patients in families A, B and C (II.2, BII.3, and CII.1). Genomic DNA was extracted and clinical mutation analysis for eleven genes was performed using the CardioChip™ that includes MYH7, cardiac myosin binding protein c (MYBPC3), TNNT2, cardiac troponin I (TNNI3), tropomyosin 1 (TPM1), ACTC, myosin regulatory light chain (MYL2), cardiac myosin essential light chain (MYL3), lysosomal associated membrane protein 2 (LAMP2), γ subunit 5-AMP-activated protein kinase (PRKAG2) and α-galactosidase a (GLA). For Family C, genomic DNA was amplified by PCR for direct sequencing of the genes: ACTC, MYBPC3, MYH7, MYL2, MYL3, tropinin C (TNNC1), TNNI3, TNNT2 and TPM1.
Molecular analysis. Exons 32 and 33 of MYBPC3 were amplified as one amplicon from DNA from patient AII.2, ligated into a plasmid vector and then sequenced to determine phase. PCR was carried out using the following primers: MYBPC3 32F GGCCTGTCGGTACCAAGTCCTGTC and MYBPC3 33R CCGCCCGCTCTTTCCCATCTC. The PCR products were ligated using the TOPO TA Cloning Kit (Invitrogen). Three independent clones were sequenced.

This study was carried out under the approval of the University of Chicago Internal Review Board.

Results

Family A. The proband (AII.2) was a Caucasian male, born after an uncomplicated term gestation by an emergent cesarean section for low heart rate; he was intubated briefly. His birth weight was 7lb 14oz. Polycythemia was treated with two blood draws and he was discharged within four days. He was the second child of healthy non-consanguineous parents (Figure 1). At eleven days of life, he presented in cardiogenic shock with profound hypotension, anuria and acidosis. His hospital course included inotropic support that was subsequently weaned allowing him to be discharged briefly. He represented with cardiogenic shock at six weeks of age and was readmitted to the hospital. He was listed for heart transplantation. However at seven weeks of age, he went into cardiac arrest and was placed on an extracorporeal membrane oxygenation. He had a biventricular assist device placed in efforts to stabilize his cardiac status and allow time for a potential organ donor; his immediate post operative course was complicated by a large left middle cerebral artery stroke with hemorrhagic conversion. The proband expired at nine weeks of age.

Echocardiography between eleven days and nine weeks showed a fractional shortening ranging from 6.5% on initial presentation to a transient improvement with inotropic and mechanical support to 22.8%, but then with a slow decline as his heart failure progressed.
LVNC was noted on his initial echocardiogram (Figure 2), and an ECG showed biventricular hypertrophy (Figure 3). The proband’s autopsy showed LV hypertrophy with coarse trabeculations, most severe at the apex, with a LV free wall thickness of 14mm. The right ventricle was hypertrophied with the right ventricular free wall thickness of 5mm. The left and right atria were hypertrophied, each with a free wall thickness of 2-3mm. Microscopic examination revealed right ventricular and left atrium myocyte hypertrophy and disarray. The LV apex and base had interstitial fibrosis with myocyte hypertrophy and disarray. Electron microscopy showed wooly densities and disorganized cristae within the mitochondria. The sarcomeres were well organized without evidence of disarray. Minimal Z line distortion was seen. The I-bands were occasionally stretched and of uneven width. The M-bands were strikingly abnormal in that they lacked definition and were difficult to discern (Figure 4).

Genetic testing of the proband identified two mutations in MYBPC3, a gene previously not associated with LVNC. The first mutation, in exon 32 of the MYBPC3 gene was 3776delA, Q1259fs. This frameshift mutation alters the protein’s terminal 16 amino acids and leads to the addition of 55 amino acids. A second mutation was also identified in his MYBPC3 gene in exon 32, 3599T>C, L1200P. This missense mutation has not been previously reported in the literature and was not identified in over 600 ethnically-matched (Caucasian) probands. To determine whether these two mutations were in cis or trans in the proband, exons 32 and 33 were amplified as one amplicon, ligated into a vector and multiple independent clones were sequenced. This analysis demonstrated that the two mutations were not found on the same allele and thus were in trans consistent with no normal MYBPC3 protein and the microscopic absence of normal M bands in his sarcomeres (Figure 4).

The proband’s mother and father were evaluated. His father (AI.1) had a structurally normal heart by echocardiography and carried neither the exon 33 nor the exon 32 changes seen in the proband. His mother (AI.2) was in good health at age 28 and denied symptoms of shortness of breath, syncope, or cardiac complications. Echocardiography revealed an end
diastolic LV diameter of 4.9 cm and an ejection fraction of 49%. There was global left ventricular systolic dysfunction and hypertrabeculation of the left ventricular apex (Figure 2, upper right). Genetic testing revealed she had only one of the mutations found in her son, MYBPC3, 3776delA, Q1259fs. MYBPC3 have been linked to later onset HCM \(^{21,22}\), and at age 28, she had only mild findings, but notably an absence of hypertrophy.

**Family B.** The proband was an African American female, born after an uncomplicated 38 week term gestation and vaginal delivery. Her birthweight was 6lbs 11oz and her length was 21 inches. She had normal development and walked at 14 months. She was hospitalized for pneumonia at eight months with full recovery. At 3.5 years, she presented with acute heart failure. Echocardiography showed a dilated LV (end diastolic diameter of 5.4cm, normal range of 2.72-3.68cm) with reduced function and fractional shortening of 9.1%. An area of noncompaction in the lateral and posterior section of the LV was seen (Figure 2 lower left). ECG showed left atrial enlargement with T wave inversion on inferior leads (Figure 3). She was treated with an ACE inhibitor, diuretics, aldactone and \(\beta\) blocker, with clinical stabilization. Her LV function improved to a fractional shortening of 14.1% and an ejection fraction of 36.7%.

Genetic testing on the proband identified a single mutation in the *MYH7* gene in exon 12, 1106G>A, R369Q. We evaluated both parents of this child, and both her father (Bl.1) and her mother (Bl.2) did not have the exon 12 mutation, consistent with a *de novo* mutation. This missense mutation has not been reported previously in the literature. However, the R369Q mutation has been identified in two pediatric individuals with cardiomyopathy where it was identified in one patient as a *de novo* variant and thus believed to be pathogenic (personal communication, Harvard Partners Laboratories).

**Family C.** The proband (CII.1) was an African American male who was referred to our institution for evaluation of LVNC after presenting at age 55 with new onset heart failure at an
outside institution. Echocardiography showed a dilated heart with a noncompacted segment of the free wall and apex (Figure 2 lower right). The LV end diastolic diameter was 6.4cm, the ventricular septal thickness in diastole was 0.83cm, the left ventricular posterior wall thickness in diastole was 1.0cm and the LV ejection fraction was 24%. His ECG showed bradycardia and left ventricular hypertrophy (Figure 3). Genetic testing on the proband identified a single mutation in MYH7 in exon 28, 3748C>T, R1250W. This missense mutation has not been reported previously. The proband has a family history of cardiomyopathy and congestive heart failure. Two siblings (CII.2 and CII.5) were affected with dilated cardiomyopathy. The proband’s niece (CIII.3) underwent cardiac transplantation at age 11 for dilated cardiomyopathy and congestive heart failure and expired 7 years after transplant. Genetic evaluation identified the R1250W mutation identified in two of his affected deceased relatives (CII.2 and CIII.3) (Figure 1).

Discussion

Sarcomere mutations in LVNC, HCM and DCM

Sarcomere mutations can produce cardiomyopathy with reduced ventricular function and hypertrabeculation. The normal left ventricle contains two layers, a compact layer and the inner trabeculated layer. During development, this inner trabecular layer normally undergoes remodeling, and hypertrabeculation is thought to represent arrest of this normal developmental process. The diagnosis of LVNC is made by identifying a ratio between noncompacted and compacted myocardium and blood flow into the intertrabecular recesses. The patients in this study met the criteria of Jenni, however it should be noted that criteria for LVNC have been evolving. The degree to which the myocardium has not undergone normal compaction may contribute to an appearance of a thickened myocardium. However, typical HCM differs from LVNC because of the spongy nature of the myocardium, and this is visualized by blood flow into the trabecular recesses on Doppler echocardiography. LVNC may be associated with
hypertrophy or dilation and may also be associated with reduced LV function. Cardiac MR may prove most valuable in quantifying trabeculation to more clearly distinguish hypertrabeculation. A recent study supported that nonischemic DCM was more likely to be associated with hypertrabeculation compared to ischemic DCM, valvular heart disease or hypertrophy suggesting.\textsuperscript{23} Nonetheless, there is clear overlap between HCM and hypertrabeculation.\textsuperscript{24} 

\textit{MYH7, ACTC, and TNNT2} have each been implicated as genetic causes of LVNC, and these genetic studies further support a cardiomyopathy continuum of HCM and LVNC. The \textit{ACTC} gene encoding cardiac actin has an E101K mutation found multiple families, where within a family, there are individuals with HCM, LVNC and/or atrial septal defects.\textsuperscript{18} This same mutation, \textit{ACTC} E101K, was described in additional families with only LVNC.\textsuperscript{17} Thirteen different \textit{MYH7} gene mutations have been previously reported as causing LVNC to date. In reviewing this literature, the age at diagnosis predominantly occurred in adulthood, differing from the cases we now identified (Table 1). While two of the three cases reported here were pediatric, one was in a 55 year old male. It is assumed that hypertrabeculation was present throughout his lifetime, and that the decline in LV function occurred with age. However, case reports have suggested that LVNC itself may be acquired.\textsuperscript{28} Longitudinal imaging studies of those with genetic mutations associated with LVNC or hypertrabeculation are required to determine the degree to which hypertrabeculation can be acquired.

\textit{MYBPC3 as a gene for LVNC with absent M bands}

The findings in Family A demonstrate that \textit{MYBPC3} mutations should also be considered for LVNC. The proband in Family A carried two different \textit{MYBPC3} mutations \textit{in trans} whereas his mother carried only one and she was mildly affected. The exon 33 mutation, 3776delA, Q1259fs appears was present in both the child and mother, and the mother at the age of 28 was only mildly affected with decreased LV function and an absence of hypertrophy. The second mutation, L1200P, was not present in either parent, and the two mutations carried
on two alleles are consistent with no normal MYBPC3 protein. MYBPC3 gene mutations cause HCM, and compound heterozygous MYBPC3 mutations with HCM have been identified displaying a more severe presentation of neonatal hypertrophic cardiomyopathy. HCM in these affected individuals showing biventricular thickening, reduced cardiac function, and histopathology showing myofibrillar disarray and interstitial fibrosis.

Microscopic findings with LVNC can overlap with those in HCM and may include subendocardial fibrosis/fibroelastosis, myocardial fibrosis, myocardial disorganization, myocardial hypertrophy and degeneration, scarring of the myocardium or signs of inflammation. The histopathology findings identified in this proband are consistent with both HCM and LVNC. The ultrastructure reported was striking for its absence of M bands. Interestingly, electron microscopic analysis of animal models of MYBPC3 gene mutations suggests that defective M lines are a feature of MYBPC3 mutations. Hearts from mice homozygous for an MYBPC3 deletion allele were noted to have an absent M-line. Similarly, a Drosophila model expressing a truncated human cMYBP-C in indirect flight muscles also showed M line distortion. The known association of MYBPC protein and the M line supports a role for this protein in M line maturation and may represent a specific finding associated with MYBPC3 gene mutations.

Clinical genetic testing for cardiomyopathy

Clinical genetic testing in cardiomyopathy is rapidly evolving. This study relied on an array-based test that allowed the study of 11 genes simultaneously. Given that over 50 genes have been associated with genetic forms of cardiomyopathy, newer technologies that permit higher throughput sequencing will allow analysis of many more genes. Other genes beyond those encoding sarcomere proteins have been associated with LVNC, notably TAZ and DTNA as well as LMNA. The TAZ gene is found on the X-chromosome and mutations associate with neutropenia. The patterns of inheritance and the absence of neutropenia make
this diagnosis less likely in the patients presented herein. Sequential gene testing is one strategy for genetic diagnosis but this approach would not identify those cases where there is contribution from more than one gene. The ability to assay multiple genes simultaneously and in a more cost effective manner will increase diagnostic yield but will also lead to the identification of benign polymorphisms that can complicate interpretation. Our study suggests that evaluating the sarcomere genes in LVNC is valuable diagnostically for the proband as well as for family members. While cardiomyopathy in this pediatric age group should likely prompt genetic testing regardless of the presence of hypertrabeculation, guidelines are only just now emerging supporting this approach. Genetic evaluation in pediatric cardiomyopathy is warranted and focus on sarcomere genes is supported by other investigations. As newer sequencing modalities become even more cost effective, recommendations for clinical genetic testing in cardiomyopathy will likely evolve.

Hypertrabeculation, cardiomyopathy, LVNC and risk assessment

Genetic evaluation is highly useful for identifying at risk relatives, or more importantly, excluding risk. Those with LVNC are likely to have similarly affected family members, and screening should be considered to detect asymptomatic family members with LVNC since they may have an increased risk of thromboembolic events or arrhythmias. In one study, 51% of LVNC probands were reported to have a family history of DCM or LVNC. Other studies have suggested that familiality of LVNC is lower, with as few as 3\%, 9\% and 12\% of LVNC probands having affected family members. The familial incidence likely varies depending on the population, the screening modalities, and the criteria used to define LVNC. The clinical overlap of LVNC with other forms of cardiomyopathy should also be considered when evaluating family members. Neonatal and childhood onset of the proband may indicate a \textit{de novo} mutation where other family members would not be affected; and in this setting, genetic testing can be highly effective to help determine risk of disease or absence thereof.
Alternatively, more than one mutation, as described here, may explain more phenotypic variability.

A recent study identified hypertrabeculation in as many as 23.6% of individuals with heart failure. A recent study identified hypertrabeculation in as many as 23.6% of individuals with heart failure. Whether these echocardiographic features identify those subjects in whom family screening or genetic testing is indicated has not been determined. LVNC criteria have been debated, and this debate complicates the sole use of these criteria in determining need for genetic evaluation. Our findings suggest that genetic testing should be considered for individuals with LVNC and cardiomyopathy with reduced LV function, since genetic information may be useful in assessing risk to family members. Together, these findings reinforce the argument that a normally functioning sarcomere is required during cardiac development and the process of LV compaction.

Conflict of interest Disclosures
None.

Funding Sources
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References


Table 1. Sarcomere mutations in LVNC.

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AS, atrial septal; ASD, atrial septal defect; AV, atrioventricular; HCM, hypertrophic cardiomyopathy; NSVT, nonsustained ventricular tachycardia; VSD, ventricular septal defect
Figures and Legends

Figure 1. Pedigrees. Solid symbols indicate a clinical diagnosis of LVNC in the setting of clinical heart failure; open symbols, normal cardiac dimensions; shaded symbols, intermediate phenotype. Gene and gene mutation are listed. Mutation positive (+), mutation negative (-). The absence of a + or - indicates that mutation status was not determined. In family A, 2-II had both mutations on two separate chromosomes while the mother carried only one mutation.

Figure 2. Echocardiographic images. A, Four chamber view showing the noncompaction of the LV (individual A-II.2). B, View of the LV showing mild hypertrabeculation in the mother of individual A-II.2) who carried only a single mutant MYBPC3 allele. Maximal wall thickness (septal and LV free wall) was 1.0 cm. C, Four chamber view showing an area of noncompaction in the lateral and posterior section of the LV (individual B-II.3). D, Four chamber view showing noncompaction of the LV (individual C-II.1).


Figure 4. Absent M bands from homozygous MYBPC3 mutations in LVNC. Electron microscopy showing findings from individual A-II.2). A control from a 9 month old individual is shown on the top and indicates a defined M band structure. The lower panel is from A-II.2 and shows some Z band disorganization as well as loss of a defined M band structure. Bar equals 0.5 μm.
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