Sarcomere Gene Mutations in Isolated Left Ventricular Noncompaction Cardiomyopathy Do Not Predict Clinical Phenotype

Running title: Probst et al.; Sarcomere Gene Analysis in Noncompaction Cardiomyopathy

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Journal Subject Codes: [16] Myocardial cardiomyopathy disease, [41] Pediatric and congenital heart disease, including cardiovascular surgery, [89] Genetics of cardiovascular disease
Abstract:

Background - Left ventricular noncompaction of the myocardium (LVNC) has been recognized as a cardiomyopathy with a genetic etiology. Mutations in genes encoding sarcomere proteins were shown to be associated with LVNC. We have evaluated the potential clinical impact of genetic analysis of sarcomere genes in patients with LVNC.

Methods and Results - In this report, we identified 5 mutations in cardiac myosin-binding protein C (MYBPC3) and 2 mutations in α-tropomyosin (TPM1) in a cohort of unrelated adult probands with isolated LVNC. The mutations in MYBPC3 and TPM1 and in 6 other previously reported sarcomere genes in this cohort resulted in a total of 18 heterozygous mutations in 63 probands (29%). β-myosin heavy chain (MYH7) was the most prevalent disease gene and accounts for 13% of cases followed by MYBPC3 (8%). Comparing sarcomere mutation-positive and mutation-negative LVNC probands showed no significant differences in terms of average age, myocardial function, presence of heart failure or tachyarrhythmias at initial presentation or at follow-up. Familial disease was found in 16 probands of which 8 were sarcomere mutation-positive. Non-penetrance was detected in 2 of 8 mutation-positive families with LVNC.

Conclusions - Mutations in sarcomere genes account for a significant proportion of cases of isolated LVNC in this cohort (29%). The distribution of disease genes confirms genetic heterogeneity and opens new perspectives in genetic testing in patients with LVNC and their relatives at high risk of inheriting the cardiomyopathy. The presence or absence of a sarcomere gene mutation in LVNC cannot be related to the clinical phenotype.

Key words: cardiomyopathy, genetics, noncompaction, sarcomere
Introduction

Left ventricular noncompaction of the myocardium (LVNC) has been recognized as a distinct primary cardiomyopathy with a genetic etiology by the American Heart Association whilst it is still considered an unclassified cardiomyopathy according to the European Society of Cardiology (ESC). LVNC is characterized by a unique myocardial morphology, hypertrophic segments that consist of a thin compacted epicardial layer and a thick noncompacted endocardial layer. The noncompacted layer contains numerous prominent trabeculations and deep intertrabecular recesses. Variable clinical features include both asymptomatic and symptomatic patients with the triad of congestive heart failure, thrombembolic events, and arrhythmias including sudden cardiac death. Based upon the variation in clinical assessment of family members familial cases were found with a frequency between 18% and 64%. As in other cardiomyopathies, index cases represent the most severe spectrum of the disease. Milder and asymptomatic forms and a more favorable prognosis of the disease were identified during family screening. In addition to reports on some very rare disease genes mutations in genes encoding sarcomere proteins were identified in a significant proportion of LVNC patients. Heterozygous mutations in genes encoding β-myosin heavy chain (MYH7), α-cardiac actin (ACTC1), and cardiac troponin T (TNNT2) account for 17% of cases of isolated LVNC in adult patients. In a study by Hoedemakers et al., LVNC was associated with genetic variants in 11 of 17 genes; 6 sarcomere, 2 calcium-handling and other cardiomyopathy genes such as lamin A/C (LMNA), ZASP (LDB3), and Tafazzin (TAZ). Mutations in the genes encoding cardiac myosin-binding protein C (MYBPC3), alpha-tropomyosin (TMP1), and cardiac troponin I (TNNI3) further supported the concept that sarcomere genes are associated with LVNC. Nevertheless, except for one report mutation screening efficiency of sarcomere genes is uncertain and penetrance and genotype/phenotype correlations in LVNC are unknown. In
this report we investigated the prevalence, distribution of disease genes, and the spectrum of mutations in our systematic study of 8 sarcomere disease genes in 63 adult patients with isolated LVNC. We extended our previous mutation analyses to MYBP3 and TMP1. The purpose of this study was a combined genetic and phenotypic analysis of this LVNC cohort to compare mutation-positive versus mutation-negative probands for possible differences in their clinical phenotypes.

**Methods**

**Clinical Evaluation**

Unrelated adult patients were recruited at two tertiary referral centers (University Hospital Zürich, Switzerland and the German Heart Institute Berlin, Germany). Informed consent was obtained from all participants according to institutional guidelines. Probands and available family members were evaluated by history taking, review of medical records, physical examination, 12-lead electrocardiography, 24h Holter monitoring, and transthoracic echocardiography. Neuromuscular abnormalities were excluded by physical examination. The diagnosis of isolated LVNC was made by echocardiography based on the presence of the established criteria by Jenni et al.\(^3\) Echocardiographic studies were performed/reviewed by two independent observers (RJ and EO).

**Mutation Screening**

Mutation screening was carried out with genomic DNA samples from 63 probands as described previously.\(^18\) Denaturing high performance liquid chromatography (DHPLC) analysis of TPM1 and MYBPC3 was performed on the WAVE® Nucleic Acid Fragment Analysis System (model 3500HT, Transgenomic, UK) and samples with an abnormal peak were sequenced as described previously.\(^8\) 360 control chromosomes were screened for the
absence of a sequence variation to recognize common polymorphisms. The allele frequency of the putative mutation in the control population was assumed to be <0.003 given that 360 control chromosomes were free of the mutation. Mutations were not reported as variants in the 1000genomes project (www.1000genomes.org; december 2010 release of 1000G which is based on the 2010 08 04 sequence and alignment release).

**Statistical Analysis**

Statistical analyses were performed with SPSS for Windows 15.0 (SPSS Inc, Chicago, III). Mann Whitney test analysis was used for categorical variables. Unpaired Student t test analysis was used for continuous variables; a normal distribution of the data was confirmed by the Kolmogorov-Smirnov-test. Descriptive data for continuous variables are presented as mean ± 1 standard deviation. P values <0.05 were considered to be significant. Probability of the event-free rate for the combined end point of death or heart transplantation was calculated by the Kaplan-Meier method of life table estimation.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

**Results**

A cohort of 63 unrelated Caucasian individuals of western European descent (43 men and 20 women; mean age at diagnosis, 40.2 +/- 15.6 years; range 15 to 70 years) underwent genetic screening of 8 sarcomere genes. The diagnosis in family members with LVNC was ascertained at an age range from 6 weeks to 73 years. Results of a subset of 6 sarcomere genes, MYH7, ACTC1, TNNT2, TNNI3, MYL2 and MYL3 in this cohort have previously been reported. In this report, heterozygous mutations in the same cohort were identified for 2 other sarcomere genes, in 5 probands in MYBPC3 and in 2 probands in TPM1 (Figure 1).
Clinical phenotypes were assessable in 4 families of the 7 probands with MYBPC3 and TPM1 mutations and familial disease was found in 2 of them (Figure 1A). Table 1 shows the mutations of probands.\(^{21-29}\) The clinical characteristics of affected family members at initial diagnosis are presented in Table 2. A comparison of the clinical characteristics between sarcomere mutation-positive and mutation-negative probands was performed (Table 3).

**Mutations in alpha-tropomyosin**

Two novel mutations were found in TPM1. A Glu192 Lys missense mutation in exon 6 of TPM1 (Figure 1B) was detected in proband KR who suffered from sudden chest pain and dyspnea at initial diagnosis. The echocardiogram revealed pronounced LVNC and increased right ventricular trabeculations. The mutation affects a glutamic acid with a high degree of conservation throughout evolution suggesting its functional importance (Figure 1B). Cardiac magnetic resonance imaging (MRI) revealed normal LV mass and extensive diffuse fibrosis of the LV (Figure 2A, 2B). Fibrosis was predominantly located on the epicardial side of the LV myocardium and extended transmurally into the anterior and inferior LV wall. The hypertrophic interventricular septum was spared by fibrosis and appeared without recesses or prominent trabeculations. This unusual pattern of fibrosis is not seen in HCM, myocardial infarction or myocarditis. Family history revealed that his father had died from heart disease at the age of 60 and his uncle from sudden cardiac death at the age of 40. Echocardiography of his son, who did not carry the TPM1 mutation showed normal LV morphology and function.

A novel Lys248Glu missense mutation was identified in exon 8 in the proband of family LVNC-117, individual I-1. The patient presented with congestive heart failure at the age of 63 years (Figure 2C, 2D). His two affected, asymptomatic children, a 32 year-old daughter (II-2) and a 34 year-old son (II-3) were only identified by family screening (Figure
1A). The daughter of individual II-2, individual III-1, was suspected to suffer from
congestive heart failure because of myocarditis several years before description of this family
history of cardiomyopathy. She was diagnosed with DCM without signs of LVNC; her
echocardiogram performed at the age of 6 weeks showed biventricular impaired systolic
function. At the age of 5 years she received a left ventricular assist device and was
successfully transplanted 3 months later. A diagnostic myocardial tissue sample taken from
the LV apex revealed pronounced endomyocardial fibroelastosis and minimal interstitial
fibrosis. Mutation analysis in individual III-1 of all coding exons of the 8 sarcomere genes
revealed the Lys248Glu mutation in \textit{TPM1} but no additional mutation in any of the genes
tested.

\textbf{Mutations in cardiac myosin-binding protein C}

In \textit{MYBPC3}, 5 mutations were identified, 4 of them were described before in
individual patients with HCM (Figure 1C). Proband AY presented with atypical chest pain
and a Gly5Arg sequence variation in exon 2 was detected in this otherwise asymptomatic
patient. This mutation was also present in his 47 year-old unaffected mother and has been
reported as a compound heterozygous mutation in a patient with HCM. Two probands,
individual I-1 of LVNC-102 and LD, carry the same missense mutation (Gly490Arg) in exon
18. The proband of LVNC-102 was first diagnosed at the age of 70 years because of dyspnea.
Family screening revealed that his asymptomatic 32-year old son was also affected. Proband
LD was diagnosed because of unclear syncope. In the two individuals with the Gly490Arg
mutation, the apex was not affected but only the midventricular inferior and lateral wall
(Figure 2E). Proband SN presented with decompensated congestive heart failure. In this
patient the Pro873Leu substitution in exon 27 is a novel mutation. Proband NS had non-
sustained ventricular flutter at electrophysiological investigation and received an ICD (Figure
2F). A 2-basepair deletion Pro955ArgfsX95 was detected in her and one of her unaffected daughters (14 years-old) that lead to an amino acid frameshift and a premature stop codon resulting in a predicted truncated protein. Variants Pro955ArgfsX95 and Gly5Arg represent the only 2 cases with familial nonpenetration in our series.

**Genetic and phenotypic analysis of cohort**

63 unrelated probands diagnosed with LVNC underwent genetic screening of 8 sarcomere genes. Eighteen mutations were identified which accounts for 29% of all probands with isolated LVNC. Fifteen distinct heterozygous mutations were found in 5 sarcomere protein genes: MYH7, MYBPC3, TPM1, ACTC1, and TNNT2. No mutations were found in TNNT2, MYL2 and MYL3. Mutations occurred most frequently in MYH7 and MYBPC3 with 13% and 8%, respectively. The frequency of mutations in the 3 other disease genes were: 3% in TPM1, 3% in ACTC1 and 2% in TNNT2. Eight of the 15 distinct heterozygous mutations were novel mutations: 6 in MYH7, 1 in TPM1 and 1 in MYBPC. The majority of mutations were missense mutations (13/18) but 3 splice-site mutations, and 2 deletions, 1 with and 1 without producing a frameshift were identified. Probands did not carry multiple sarcomere mutations. Familial disease was present in 16 probands of which 8 patients were mutation-positive and 8 were mutation-negative for sarcomere genes.

A phenotypic comparison between the 18 mutation-positive and 45 mutation negative-patients was performed (Table 3). Both groups did not differ in sex, age at diagnosis or age at follow-up. The duration of follow-up was slightly shorter in the mutation-negative group (p<0.046). The following criteria were also not different between the 2 groups at the time of diagnosis: (i) number of symptomatic versus asymptomatic patients (dyspnea, syncope, shock, palpitations), (ii) tachyarrhythmias (atrial flutter/fibrillation, AV nodal reentrant tachycardia, ventricular tachycardia, ventricular flutter/fibrillation), and (iii) heart failure.
Echocardiographic criteria also were indistinguishable between the 2 groups: LV enddiastolic diameter (LVEDD), LV fractional shortening (LV-FS), LV ejection fraction (LV-EF) at diagnosis and at follow-up, respectively. The LV wall was divided into nine segments to describe the distribution of affected LVNC segments in the 2 groups: the whole apex (one segment); at the base and at the midventricular level, the LV was divided into 4 segments each (inferior, lateral, anterior and septal). The number of affected segments was comparable in both groups (mean was 3 affected segments each). The most prevalent locations of LVNC were (mutation negative/mutation-positive): apex (88%/88%), midventricular lateral wall (82%/81%), and midventricular inferior wall (76%/88%). Only the midventricular septum was more frequently affected in the mutation-positive group (8%/31%; p<0.046), but no differences in the distribution of the other 8 segments were found between the 2 groups.

There was no difference between the two groups with regard to the number of implantations of an automated cardiac defibrillator. Patients that received a cardiac transplant or died were more likely to be found in the mutation-negative group (p<0.048). However, the Kaplan-Meyer survival curve for the combined end-point heart transplantation or death did not demonstrate any difference between the two groups.

**Discussion**

The prevalence, distribution of disease genes, spectrum of mutations and clinical features related to mutations in 8 sarcomere genes were investigated in adult patients with isolated LVNC. The combined genetic and phenotypic evaluation in this study has implications for genetic counseling and testing in LVNC as sarcomere gene mutations are an important determinant of LVNC.
Prevalence and Spectrum of Sarcomere Gene Mutations in LVNC Patients

In the total cohort studied, 18 mutations in 5 sarcomere genes were identified in 29% of adult patients with isolated LVNC. In the study by Hoedemakers et al.\textsuperscript{13} mutations in 11 genes, among them 6 sarcomere genes, in 41% of patients with LVNC were identified. In adult patients mutations in \textit{MYH7} were the most frequent genetic defects in adult patients: in this study in 8 of 63 probands (13 \%) and in the study by Hoedemaekers et al.\textsuperscript{13} in 9 of 57 probands (16\%). Therefore the known prominent role for \textit{MYH7} in cardiomyopathies, such as in HCM\textsuperscript{24} and DCM\textsuperscript{30}, is becoming evident in LVNC as well. The study by Hoedemakers et al.\textsuperscript{13} also included children and probands with non-isolated LVNC; patients with Dutch founder mutations were reported. Nevertheless, the distribution of mutations in 5 sarcomere disease genes was comparable in both cohorts, \textit{MYH7} being the most prevalent disease gene (20\%\textsuperscript{13} versus 13\% in our study), followed by \textit{MYBP3} (5\%\textsuperscript{13} versus 8\% in our study) and mutations in \textit{TNNT2, ACTC1} and \textit{TPM1} were rare and accounted for LVNC in only 1 or 2 probands in the respective cohorts. The most prevalent type of mutations in both cohorts was missense mutations (16 of 21 distinct mutations\textsuperscript{13} versus 11 of 15 distinct mutations in our study) in \textit{MYH7, MYBP3, TNNT2, ACTC1}, and \textit{TPM1}. In addition to 18 probands with single mutations Hoedemakers et al.\textsuperscript{13} described 4 probands with compound or double heterozygote mutations and 1 proband had 3 mutations. In contrast, all 18 mutation-positive probands in our study carried single mutations.

Although 8 of the 15 distinct mutations were novel in our cohort it can be anticipated that they are not specifically found in LVNC. 7 of the 15 distinct mutations have been described before in apical HCM, DCM and HCM. In our previous study\textsuperscript{18}, the Arg243His substitution in \textit{MYH7} had been found in a patient with apical HCM\textsuperscript{27}, the Glu101Lys exchange in \textit{ACTC1} in apical HCM and LVNC\textsuperscript{27,28}, and the Arg131Trp substitution in \textit{TNNT2} in an individual with DCM\textsuperscript{29}. In this study, the exchange Glu192Lys in TPM1 has
been reported before in a patient with HCM and atrial septal defect (http://www.cardiogenomics.org). The MYBPC3 substitutions Gly5Arg and Gly490Arg were found in individuals with HCM. The Pro955Arg fsX95 frameshift mutation has been reported before in 4 individuals with HCM (3 single mutations, 1 compound heterozygous mutation). The MYBPC3 Pro873Leu substitution is a novel mutation, a Pro873His change has been reported as a homozygous and as a compound heterozygous mutation. Therefore, a direct relationship between specific mutations and specific cardiomyopathy phenotypes could not be established.

**Penetrance and Variable Phenotypes in LVNC**

The penetrance of a mutation is defined as the percentage of mutation carriers expressing a phenotype and most autosomal dominant cardiomyopathies are characterized by incomplete penetrance or more age-related penetrance. Variants Pro955ArgfsX95 and Gly5Arg in MYBPC3 represent the only cases with familial non-penetration in our series (2/8 probands). Thus, penetrance in our series of LVNC patients was incomplete but non-penetration affected only families with MYBPC3 mutations. Mutations in the most prominent disease gene in LVNC, MYH7, were fully penetrant. There is also variable expressivity in cardiomyopathies and there can even be large differences between relatives of the same family (intra-familial variability) who carry the same mutation. For LVNC this has been documented for one family carrying the Glu101Lys exchange in ACTC1 associated with apical HCM, LVNC, and septal defects. LVNC-117 in this series is an interesting example of two cardiomyopathy phenotypes, adult LVNC and neonatal DCM, segregating within one family and associated with the same mutation. Intrafamilial differences or a more severe or early phenotype are sometimes explained by the presence of a second causal mutation in the family. For kindred LVNC-117, individual III-1 with neonatal DCM, compound or double
heterozygosity or homozygosity in sarcomere genes was excluded and could not account for the severe phenotype.

Familial cardiomyopathy was detected in 25% of probands. Echocardiography revealed LVNC in affected family members, except for family LVNC-117 in which DCM was present in one neonate. The number of familial cases in this study is relatively low compared to the study by Hoedemaekers et al.\textsuperscript{13} with a detection of familial cardiomyopathy in 64% of probands due to the almost complete echocardiographic workup of their families. In their study 44% of familial disease would have remained undetected by ascertainment of family history alone without clinical cardiac family screening. One limitation of our study was the incomplete clinical family screening for which position statements have recently been published.\textsuperscript{20,31} Clinical and echocardiographic screening should be considered to detect asymptomatic family members with LVNC because of the potentially increased risk for arrhythmias, myocardial dysfunction and/or thrombembolic events. In addition, genetic evaluation is highly useful for identifying other at-risk relatives without the morphological changes as the penetrance of LVNC may be incomplete in some cases. The duration of follow-up was shorter in the mutation-negative group and patients that received a cardiac transplant or died were more likely to be found in the mutation-negative group. Therefore the mutation-positive group seems to have a slightly more benign clinical phenotype. Long-term studies are needed to detect possible relationships between the type of mutation, genetic modifiers and clinical phenotype.

**LVNC: a defect of cardiomyocyte dysfunction during myocardial development?**

How mutations in sacromere genes could have detrimental effects on cardiac morphogenesis is unclear. LVNC is still considered an unclassified cardiomyopathy by the ESC\textsuperscript{2} and other investigators\textsuperscript{32,33} who suggest that it is not clear whether it is a separate
cardiomyopathy or merely a morphological trait shared by many phenotypically distinct cardiomyopathies. Biagini reported a series of LVNC patients in which patients fulfilled echocardiographic criteria for DCM, HCM and restrictive cardiomyopathy. The causal role of noncompaction in the pathogenesis of cardiomyopathy was recently questioned by transgenic mice carrying a human troponin T mutation leading to LVNC in patients.

Not much is known about the molecular regulation of ventricular trabeculation. Early myocardial development is characterized by a subdivision of the myocardial wall into an outer highly mitotic compact zone and an inner trabecular zone with much less proliferative activity. A correct balance between proliferation and differentiation of cardiomyocytes seems to be critical in ventricular chamber formation. In zebrafish Aumann and colleagues demonstrated that chamber morphology develops via changes in cell morphology. Their model suggests that even subtle changes of circulation or contractility caused by mutated sarcomere genes could lead to abnormalities in cell morphology and consequences for chamber morphology. How this model could account for the localized remodeling of the ventricular apex, which is primarily affected in LVNC, remains to be determined.

In this study several mutations in MYBPC3 were found in LVNC. In HCM, most MYBPC3 mutations are predicted to produce C-terminally truncated proteins, lacking titin and/or major myosin binding sites. Recent work strongly argues for haploinsufficiency as the disease mechanism for both, truncation and missense MYBPC3 mutations. The sarcomeric phenotype in HCM with MYBPC3 mutations includes a primary contractile sarcomeric defect causing deranged secondary alterations in protein phosphorylation. It remains to be determined if contractile dysfunction is the pivotal link between mutant sarcomeric protein and the pathological morphology observed in LVNC.

**Conclusions**
In this cohort of adult patients with isolated LVNC mutations in sarcomere genes have a prominent role with a prevalence of 29%. Of the 8 genes tested, MYH7 and MYBPC3 are the most frequently mutated sarcomere genes in LVNC, 13% and 8% respectively. Sarcomere mutation-positive probands cannot be distinguished from mutation-negative probands on the basis of their clinical phenotypes. However, genetic testing of sarcomere genes is a valuable diagnostic tool for the probands and their relatives at high risk of inheriting the cardiomyopathy.

Acknowledgments: We thank Sigrid Milan for excellent technical expertise.

Funding Sources: This work was supported by a grant from the Deutsche Forschungsgemeinschaft (SA 1389/1-1) to Dr. Klaassen.

Conflict of Interest Disclosures: None

References:


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Table 1. Mutations of 63 LVNC Probands

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<th>Protein</th>
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<td>p.Glu192Lys</td>
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<td>p.Lys248Glu</td>
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<td>LVNC-102</td>
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<td>p.Pro955ArgfsX95</td>
<td>28</td>
<td>This study; [21]; [24,25,26] compound heterozygous with Glu258Lys</td>
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|          |      |            |         |      |           |
| **Mutations previously published by Klaassen et al. [18]** |      |            |         |      |           |
| *MYH7*  |      |            |         |      |           |
| LVNC-101| II-4 | c.818+1G>A | p.Arg243His | 18  | [18,27] |
| LVNC-107| II-3 | c.814G>A   | p.Arg243His | 18  | [18,27] |
| LVNC-108| II-2 | c.818+1G>A | p.Arg243His | 18  | [18,27] |
| LVNC-109| II-1 | c.818+3G>C | p.Arg243His | 18  | [18,27] |
| Sporadic | AJ   | c.801 _803delGAC | p.Asp239del | 8   | [18,27] |
| Sporadic | SD   | c.840T>C   | p.Phe252Leu | 9   | [18,27] |
| Sporadic | IP   | c.4161C>T  | p.Arg1359Cys | 30  | [18,27] |
| Sporadic | MT   | c.5382G>A  | p.Ala1766Thr | 37  | [18,27] |
| *ACTC1* |      |            |         |      |           |
| *TNN1*  |      |            |         |      |           |
| Sporadic | CR   | c.450C>T   | p.Arg131Trp | 10  | [18,29] |

Sporadic, only the proband of a family was affected with LVNC; LVNC-101, -102, -107, -108, -109, -110, -111, -117, familial disease, apart from the proband at least one more affected individual with LVNC in a family.

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**Table 2. Clinical Characteristics of Affected Family Members at Initial Diagnosis**

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<th>ID</th>
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<td>3</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>II-1</td>
<td>32/M</td>
<td>1</td>
<td>3</td>
<td>0.4</td>
</tr>
<tr>
<td>Sporadic</td>
<td>LD</td>
<td>24/F</td>
<td>1</td>
<td>3</td>
<td>0.5</td>
</tr>
<tr>
<td>Sporadic</td>
<td>SN</td>
<td>37/M</td>
<td>4</td>
<td>2</td>
<td>3.0</td>
</tr>
<tr>
<td>Sporadic</td>
<td>NS</td>
<td>28/F</td>
<td>2</td>
<td>2</td>
<td>1.7</td>
</tr>
</tbody>
</table>

* Noncompacted segments: no=0; apex=1; apex, midventricular wall=2; midventricular wall=3; LVEDD, left ventricular enddiastolic diameter; Z score, normal reference range -2 < +2; EF/FS, left ventricular ejection fraction/fractional shortening; AF, atrial fibrillation; CHF, congestive heart failure; PHT, pulmonary hypertension; ICD, intracardiac defibrillator; NSVT, nonsustained ventricular tachycardia; HTX, heart transplantation; EPI, electrophysiologic investigation; Sporadic, only the proband of a family was affected with LVNC; LVNC-102, -117, familial disease, apart from the proband at least one more affected individual with LVNC in a family.

**Table 3. Comparison of Probands with and without Sarcomere Gene Mutations**

<table>
<thead>
<tr>
<th></th>
<th>Mutation-positive</th>
<th>Mutation-negative</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex M/F, n</td>
<td>13/5</td>
<td>30/15</td>
<td>NS</td>
</tr>
<tr>
<td>Age (D), y</td>
<td>39.2±16.9</td>
<td>43.3±15.0</td>
<td>NS</td>
</tr>
<tr>
<td>Age (FU), y</td>
<td>45.5±16.6</td>
<td>46.8±15.7</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of FU, y</td>
<td>6.7±5.5</td>
<td>3.8±4.2</td>
<td>0.046</td>
</tr>
<tr>
<td><strong>At diagnosis (D)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac symptoms, n (%)</td>
<td>14 (78)</td>
<td>40 (89)</td>
<td>NS</td>
</tr>
<tr>
<td>Heart failure, n (%)</td>
<td>10 (56)</td>
<td>28 (62)</td>
<td>NS</td>
</tr>
<tr>
<td>Tachyarrhythmias, n (%)</td>
<td>2 (11)</td>
<td>7 (16)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Echocardiography</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVEDD (D/FU, y)</td>
<td>60.0±9.2/59.7±9.0</td>
<td>60.0±12.3/58.4±10.2</td>
<td>NS</td>
</tr>
<tr>
<td>LV-FS (D/FU, y)</td>
<td>20.0±9.3/22.2±10.3</td>
<td>22.9±8.9/23.4±11.1</td>
<td>NS</td>
</tr>
<tr>
<td>LV-EF (D/FU, y)</td>
<td>36.1±17.1/39.6±14.4</td>
<td>38.7±16.1/42.3±15.1</td>
<td>NS</td>
</tr>
<tr>
<td><strong>At follow-up (FU)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICD, n (%)</td>
<td>3 (17)</td>
<td>7 (16)</td>
<td>NS</td>
</tr>
<tr>
<td>Alive /HTX or death, n (%)</td>
<td>16 (89)/2 (11)</td>
<td>36 (80)/9 (20)</td>
<td>0.048</td>
</tr>
</tbody>
</table>

**At diagnosis (D) /at follow-up (FU)** Cardiac symptoms (dyspnea, syncope, shock, palpitations); Heart failure (NYHA II-IV); Tachyarrhythmias (atrial flutter/fibrillation, AV nodal reentrant tachycardia, ventricular tachycardia, ventricular flutter/fibrillation); ICD, intracardiac defibrillator; LVED, left ventricular enddiastolic diameter; EF/FS, left ventricular ejection fraction/fractional shortening; HTX, heart transplantation; Values are given as mean ± standard deviation; * from 18 mutation-positive probands 16 were alive and 2 were deceased or had received a cardiac transplant, from 45 mutation-negative probands 36 were alive and 9 were deceased or had received a cardiac transplant.
**Figure Legends:**

**Figure 1.** A. Pedigrees of kindreds LVNC-102 (mutation in MYBPC3, Gly490Arg) and LVNC-117 (mutation in TPM1, Lys248Glu) with autosomal dominant LVNC. Filled symbols indicate individuals with LVNC; open symbols, unaffected status; shaded symbols, unknown clinical status. Plus signs (+) indicate the presence of a mutation, and minus signs (-) the absence of a mutation. B. Alignment of the regions flanking the novel mutations in TPM1 showing evolutionary conservation of the mutated residues across species. The residues with the novel amino acid changes in LVNC are boxed. Dots identify amino acids identical to the one in the human sequence. C. Schematic domain structure of cardiac myosin binding protein -C (cMYBP-C) protein with the location of mutations in the encoding gene MYBPC3. cMYBP-C consists of 8 immunoglobulin (Ig)-like and 3 fibronectin (Fn3) domains with binding sites for myosin and titin. The N-terminal C0 is specific for cMYBP-C. The cardiac-specific phosphorylation sites are located between C1 and C2. The MYBPC3 missense mutations were found in different regions of the molecule. The C1 deletion in exon 28 (c.2919-2920delC1) creates a frameshift resulting in a premature termination codon in exon 30.

**Figure 2.** A, B Magnetic resonance images, short axis orientation at midventricular level, of individual KR with a Glu192 Lys mutation in TPM1. A, SSFP image: thin epicardial and trabeculated endocardial layer of the myocardium. B, Late gadolinium enhancement image: scar tissue (bright region) in the heavily trabeculated areas (anterior, inferior) while the septum and parts of the lateral wall are not affected (black). C-F Echocardiographic images of affected individuals with LVNC. C, Enddiastolic parasternal short axis and D, enddiastolic apical 4-chamber view of individual I-1 of kindred LVNC-117. E, Enddiastolic parasternal short axis view of individual LD with midventricular LVNC. F, Enddiastolic 4-chamber view of proband NS with apical and midventricular LVNC.
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Susanne Probst, Erwin Oechslin, Pia Schuler, Matthias Greutmann, Philipp Boyé, Walter Knirsch, Felix Berger, Ludwig Thierfelder, Rolf Jenni and Sabine Klaassen

_Circ Cardiovasc Genet_. published online May 6, 2011;
_Circulation: Cardiovascular Genetics_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1942-325X. Online ISSN: 1942-3268

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