The Importance of Genetic Counseling, DNA Diagnostics, and Cardiologic Family Screening in Left Ventricular Noncompaction Cardiomyopathy

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Background—Left ventricular (LV) noncompaction (LVNC) is a distinct cardiomyopathy featuring a thickened bilayered LV wall consisting of a thick endocardial layer with prominent intertrabecular recesses with a thin, compact epicardial layer. Similar to hypertrophic and dilated cardiomyopathy, LVNC is genetically heterogeneous and was recently associated with mutations in sarcomere genes. To contribute to the genetic classification for LVNC, a systematic cardiological family study was performed in a cohort of 58 consecutively diagnosed and molecularly screened patients with isolated LVNC (49 adults and 9 children).

Methods and Results—Combined molecular testing and cardiological family screening revealed that 67% of LVNC is genetic. Cardiological screening with electrocardiography and echocardiography of 194 relatives from 50 unrelated LVNC probands revealed familial cardiomyopathy in 32 families (64%), including LVNC, hypertrophic cardiomyopathy, and dilated cardiomyopathy. Sixty-three percent of the relatives newly diagnosed with cardiomyopathy were asymptomatic. Of 17 asymptomatic relatives with a mutation, 9 had noncompaction cardiomyopathy. In 8 carriers, nonpenetration was observed. This may explain that 44% (14 of 32) of familial disease remained undetected by ascertainment of family history before cardiological family screening. The molecular screening of 17 genes identified mutations in 11 genes in 41% (23 of 56) tested probands, 35% (17 of 48) adults and 6 of 8 children. In 18 families, single mutations were transmitted in an autosomal dominant mode. Two adults and 2 children were compound or double heterozygous for 2 different mutations. One adult proband had 3 mutations. In 50% (16 of 32) of familial LVNC, the genetic defect remained inconclusive.

Conclusion—LVNC is predominantly a genetic cardiomyopathy with variable presentation ranging from asymptomatic to severe. Accordingly, the diagnosis of LVNC requires genetic counseling, DNA diagnostics, and cardiological family screening. (Circ Cardiovasc Genet. 2010;3:232-239.)

Key Words: noncompaction ▪ cardiomyopathy ▪ family study ▪ genetics ▪ hypertrophy ▪ ventricles

Left ventricular noncompaction (LVNC) is a cardiomyopathy featuring segmental thickening of the LV wall with a thin, compact, epicardial layer and an excessively thickened endocardial layer with prominent, deep intertrabecular recesses. Application of the echocardiographic diagnostic criteria for LVNC as postulated by Jenni et al together with advances in cardiological imaging techniques have enhanced awareness and diagnosis of LVNC. Consequently, LVNC was incorporated in the most recent classification of cardiomyopathies as a genetic disease. Prevalence of LVNC, estimated from retrospective studies, ranges from 4.5 to 26 per 10 000 adult patients referred for echocardiography. LVNC was diagnosed in 3.7% of patients with an LV ejection fraction ≤45%, suggesting that LVNC might not be a rare disorder in adults. In pediatric series, LVNC is the most frequent cardiomyopathy after dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM), comprising 9% of childhood cardiomyopathies. Clinical features include heart failure, arrhythmias, and thromboembolic events. Familial disease was estimated to occur in 18% to 50% of adults with isolated LVNC, mostly consistent with an autosomal dominant mode of inheritance. Intrafamilial phenotypic variability, including LVNC, HCM, and DCM, suggests that these cardio-
myopathies may be part of a broader cardiomyopathy spectrum. The first association of isolated LVNC with mutations in the cardiac β-myosin heavy chain gene (MYH7) was reported in 2 unrelated Dutch families.14 LVNC also was associated with mutations in other sarcomere genes (cardiac troponin T [TNNT2] and cardiac α-actin [ACTC1]) in 17% of 63 adult patients with LVNC.15–17 Linking LVNC to defects in the MYH7, TNNT2, and ACTC1 genes encoding sarcomere components that are frequent causes of HCM and DCM, provides additional evidence for a shared genetic susceptibility to LVNC, HCM, and DCM. Reports of mutations in lamin A/C (LMNA), α-dystrobrevin (DTNA), cypher/ZASP or lim domain binding 3 (LDB3), and sodium channel type V (SCN5A) expanded the genetic spectrum of LVNC.18–20 Other genetic causes, characteristically in complex childhood LVNC with congenital heart defects or (metabolic) syndromes, include Barth syndrome with mutations in the Tafazzin gene (TAZ)21,22 and rare chromosomal defects and loci.23–30 The present study investigates the heredity of LVNC, the spectrum of clinical features, and the genetic causes of LVNC by combining systematic cardiological family studies with extensive molecular analysis.

**Methods**

**Study Population**

The study comprised 58 unrelated patients with isolated LVNC; 53 were diagnosed consecutively from 2005 to 2008 in the cardigenetics clinic of the Erasmus MC in Rotterdam and 5 in other tertiary referral centers in The Netherlands. All fulfilled the 4 echocardiographic diagnostic Jenni criteria: (1) excessively thickened LV myocardial wall with a 2-layered structure comprising a compact epicardial layer (C) and a noncompacted endocardial layer (NC) of prominent trabeculations and deep intertrabecular recesses; (2) maximal end-systolic ratio of noncompacted to compacted wall measured at the parasternal short axis; (3) color Doppler evidence of deep perfused intertrabecular recesses; and (4) absence of coexisting
cardiac anomalies. Subsequently, all patients were referred for genetic counseling and DNA analysis and to initiate family studies, as depicted in Figure 1.

**Cardiological Family Study and Molecular Analysis**

Family studies were initiated by ascertainment of family histories and inviting initially first- and second-degree relatives for genetic counseling. When possible, “cascade screening” for cardiomyopathies was pursued. Participation of 50 families of probands allowed inclusion of 194 relatives (Table 1). Relatives were referred for cardiological screening unless a familial pathogenic mutation had been detected. In that case, only mutation-positive individuals and relatives refusing DNA testing were examined cardiologically. Informed consent was requested to review medical records from 31 deceased relatives reported to be affected. Details of the family studies of the probands identified with a genetic defect are presented in the Data Supplement.

**Cardiological Family Study**

Cardiological screening of relatives was performed by 2 cardiologists (K.C. and M.M.) and included a review of the medical history, physical examination, electrocardiography (Mortara Portrait, Milwaukee, Wis), and 2-dimensional echocardiography (IE33 system with a 5–1 transducer; Philips Medical Systems, Best, The Netherlands). If the imaging quality was poor, especially at LV apical or midventricular walls, MRI (1.5-T scanner; Signa CV/GE Medical Systems, Milwaukee, Wis) was performed (n=26). Measuring the maximal NC and C with electronic calipers in end-systolic parasternal short-axis or apical 4-chamber view assessed extent and severity of noncompaction. Relatives were diagnosed with Cardiological Family Study and Cardiological Family Screening if they fulfilled the following 3 criteria: (1) segregation with disease in a family, (2) absence on 384 ethnically matched healthy control chromosomes, and (3) likely pathogenic according to prediction software (SIFT and PolyPhen).22 DNA variants not fulfilling these criteria were considered unclassified variants.

**Statistics**

Statistical analyses were performed with SPSS for Windows 15.0 (SPSS Inc, Chicago, Ill). Unpaired Student t test analysis was used for continuous variables. Descriptive data for continuous variables were presented as mean±1 SD. χ² analysis was used for categorical variables, and ρ values <0.05 were considered to be significant. The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

**Results**

The cardiological screening of relatives and the molecular analysis of probands and relatives combined showed that 67% (39 of 58) of LVNC is genetic (Table 2). The cardiological family study identified 64% (32 of 50) of isolated LVNC as familial. Genetic defects were identified in 50% (16 of 32) of cardiologically confirmed familial LVNC. In 50% (16 of 32) of familial disease, the genetic defect remained unknown. With extensive DNA screening, we found a mutation in 41% (23 of 56) of all tested probands. These results clearly indicate the importance of combining cardiological analysis, and no DNA was available from 1 patient who died at 11 days of age. The parents of this patient were cardiologically unaffected and did not have a mutation.

The mutations previously associated with cardiomyopathy (LVNC or HCM) were regarded as pathogenic. Novel mutations were considered to be pathogenic when they were truncating, splice-site, or de novo mutations or if they fulfilled the following 3 criteria: (1) segregation with disease in a family, (2) absence on 384 ethnically matched healthy control chromosomes, and (3) likely pathogenic according to prediction software (SIFT and PolyPhen).22 DNA variants not fulfilling these criteria were considered unclassified variants.

**Table 1. Descriptives of the LVNC Family Study**

<table>
<thead>
<tr>
<th>Category</th>
<th>Total</th>
<th>Men</th>
<th>Women</th>
<th>Mean Years ± SD (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Probands</strong></td>
<td>58</td>
<td>30</td>
<td>28</td>
<td>39±17 (0 to 63)</td>
</tr>
<tr>
<td>Adults</td>
<td>49</td>
<td>26</td>
<td>23</td>
<td>44±12 (19 to 63)</td>
</tr>
<tr>
<td>Children</td>
<td>9</td>
<td>4</td>
<td>5</td>
<td>7±8 (0 to 17)</td>
</tr>
<tr>
<td>Participating relatives</td>
<td>194</td>
<td>89</td>
<td>105</td>
<td>41±21 (0 to 77)</td>
</tr>
<tr>
<td>Parents</td>
<td>40</td>
<td>20</td>
<td>20</td>
<td>55±15 (23 to 74)</td>
</tr>
<tr>
<td>Siblings</td>
<td>64</td>
<td>27</td>
<td>37</td>
<td>38±18 (3 to 66)</td>
</tr>
<tr>
<td>Children</td>
<td>41</td>
<td>22</td>
<td>19</td>
<td>23±15 (0 to 56)</td>
</tr>
<tr>
<td>Second-degree relatives</td>
<td>43</td>
<td>18</td>
<td>26</td>
<td>51±19 (15 to 76)</td>
</tr>
<tr>
<td>Third-degree and more distant relatives</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>38±1 (37 to 39)</td>
</tr>
</tbody>
</table>

**Table 2. Cardiological Family Studies and Genotyping of 58 LVNC Probands**

<table>
<thead>
<tr>
<th>Proband</th>
<th>De Novo</th>
<th>Inconclusive</th>
<th>Not Performed</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>With mutation</td>
<td>16*</td>
<td>1*</td>
<td>3*</td>
<td>23</td>
</tr>
<tr>
<td>Without mutation</td>
<td>15*</td>
<td>13</td>
<td>5</td>
<td>33</td>
</tr>
<tr>
<td>Without DNA analysis</td>
<td>1*</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>18†</td>
<td>8</td>
<td>58</td>
</tr>
</tbody>
</table>

*Genetic LVNC (total 39).
†Including the family of the de novo proband.
family screening for cardiomyopathy with genetic testing of patients with LVNC.

Family histories reported by probands before DNA testing and cardiological family studies were performed failed to identify 44% (14 of 32) of familial disease. Only 9 (53%) of the 17 adult patients with a mutation reported familial disease before DNA testing and cardiological family evaluation. Familial disease was correctly reported by 8 of 14 (57%) of the 17 adult patients with a mutation reported familial disease. 

Only 9 (53%) of the 32 relatives endorsed the pathogenicity of the mutation in families with a mutation, unaffected adult carriers of a mutation were approximately the same age as the affected relatives. Cardiological evaluation revealed that 56% of unaffected carriers were asymptomatic when diagnosed (29% and 16%; 0.2). Fifty-six percent of unaffected carriers were asymptomatic when diagnosed (29% and 16%; 0.2). In 9 children, LVNC was diagnosed: in 4 before age 1 year, in 3 between 1 to 10 years, and in 2 between 10 and 18 years. 

Similar proportions of adult probands with a single mutation and without a mutation were asymptomatic when diagnosed (29% and 16%; 0.3; Table 3). All adult patients with multiple mutations presented with New York Heart Association class II and III. These differences cannot be attributed to a selection bias because clinical diagnosis of LVNC preceded DNA testing.

In 9 children, LVNC was diagnosed: in 4 before age 1 year, in 3 between 1 to 10 years, and in 2 between 10 and 18 years. The 2 children with multiple mutations were severely affected with cerebral infarctions, and 1 had a heart transplant at age 7 years (Figure 2). All the children were the first in their families to be diagnosed with cardiomyopathy; cardiological screening and DNA testing indicated familial LVNC in 89% (8 of 9) of their families; LVNC was diagnosed in 3

**Cardiological Studies**

There was no difference in age at diagnosis in adult probands with respect to gender (P=0.4), between adults with 1 or multiple mutations and those without a mutation (P=0.4), or between the probands and affected relatives (P=0.2). In families with a mutation, unaffected adult carriers of a mutation were approximately the same age as the affected carriers (P=0.2). Fifty-six percent of unaffected carriers were older than 40 years, indicating nondependent or age-dependent penetrance of LVNC.

**Figure 2.** MYBPC3 p.Gly148Arg and c.2373dupG mutations in 1 family. Compound heterozygosity for 2 different MYBPC3 mutations in a patient with LVNC diagnosed at age 7 years from a family where 1 grandparent was previously diagnosed with HCM (Data Supplement; 23). Family studies identified a spectrum of cardiomyopathies: The asymptomatic parents were carriers, LVNC in the father and HCM in the paternal grandfather associated with the p.Gly148Arg mutation, and HCM in the maternal grandfather associated with c.2373dupG. Arrow indicates the proband; dagger, deceased. CVA indicates cerebrovascular accident; ND, not determined (ie, no cardiologic and molecular testing); SIDS, sudden infant death syndrome; CoAo, coarctation of the aorta; and HTX, heart transplant.
of 17 (18%) parents (as illustrated in Figure 3); and 3 of 15 (20%) were unaffected carriers.

In total, cardiological screening was performed in 145 first-degree, 43 second-degree, and 6 more distantly related relatives (Table 1). Of the 69 (35%) relatives diagnosed with cardiomyopathy 47 had LVNC, 5 had HCM, 15 DCM, and 2 congestive CM (Table 3). The majority (63%) of the relatives diagnosed with cardiomyopathy was asymptomatic. There was no significant difference in severe complications in affected relatives (heart failure, arrhythmia and thromboembolic events) in families without and families with a mutation (23% versus 13%; OR, 2.01; \( P = 0.36 \)). Two large families without mutation had recurrence of a severe phenotype; affected relatives in these families had been diagnosed prior to this study.

In 34% (11 of 32) of familial disease, familial aggregation of LVNC, HCM, and DCM was observed. HCM and/or DCM was diagnosed in 4 families with a mutation (Data Supplement; 8, 10, 16, and 23; Figure 2) and in 7 families without a mutation (Table 3).

### Table 3. Cardiological Features in LVNC Families

<table>
<thead>
<tr>
<th></th>
<th>Mutation*</th>
<th></th>
<th>No Mutation*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Probands (23)</td>
<td>Relatives (39)</td>
<td>Probands (35)</td>
</tr>
<tr>
<td></td>
<td>Adult (17)</td>
<td>Children (6)</td>
<td>Affected (34)</td>
</tr>
<tr>
<td>Age±SD, y</td>
<td>41±11</td>
<td>6±6</td>
<td>41±21</td>
</tr>
<tr>
<td>Men</td>
<td>8</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td>Presentation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>4</td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td>Heart failure</td>
<td>6</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Arrhythmias</td>
<td>5</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Thromboembolism</td>
<td>1</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Other‡</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>NYHA I</td>
<td>5</td>
<td>21</td>
<td>2</td>
</tr>
<tr>
<td>NYHA II</td>
<td>7</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>NYHA III</td>
<td>5</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>NC/C ratio ≥2</td>
<td>17</td>
<td>25</td>
<td>32</td>
</tr>
<tr>
<td>NC/C ratio 1.0 to 1.9</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECG abnormal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVH</td>
<td>1</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Abnormal repolarization</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal Q</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Bundle-branch block</td>
<td>4</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>AV block</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-wave inversion</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVNC</td>
<td>17</td>
<td>6</td>
<td>25</td>
</tr>
<tr>
<td>HCM</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCM</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Congestive CM</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history of CM§</td>
<td>9</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Familial screening</td>
<td></td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Familial CM</td>
<td>13</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>Congenital heart defect in relative¶</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

NYHA indicates New York Heart Association classification; NC/C ratio, ratio of noncompacted to compacted wall; LVH, left ventricular hypertrophy; and CM, cardiomyopathy.

* Families with and without mutation.
† Other includes NC/C ratio between 1.0 and 1.9 and/or ECG anomalies.
‡ Chest pain (n=4), enlarged heart on x-ray, preoperative screening, prenatal sonography, and cardiac screening before Ritalin use.
§ Before cardiological family studies.
|| Cardiological screening/DNA analysis.
¶ Ebstein malformation, Fallot tetralogy, atrial septal defect type II and ventricular septal defect, or aortic coarctation in 4 families with a mutation. Valvular pulmonic stenosis; atrial septal defect type II, ventricular septal defect, or pulmonic atresia; or patent ductus arteriosus and aortic coarctation in 4 families without mutation.
In 7 families, congenital heart malformations were diagnosed. In 1 family with an MYH7 mutation, LVNC was associated with Ebstein anomaly, and in 2 families with MYBPC3 mutations, 1 relative with the mutation had Fallot tetralogy without LVNC and 1 had an aortic coarctation but did not have a DNA test (Data Supplement; 1, 10, and 22; Figure 2). In 3 families without a mutation, LVNC occurred together with valvular pulmonic stenosis, ventricular septal defect, atrial septal defect type II, pulmonic atresia, patent ductus arteriosus, or aortic coarctation in 6 relatives.

**Discussion**

The approach of this study was to combine cardiological family studies with extensive genetic testing to establish a genetic classification of LVNC. The results showed that isolated LVNC is predominantly (67%) a genetic condition, including HCM and DCM in 11 families (34%). The molecular screening of a large number of genes in this study allowed expanding the genetic spectrum of LVNC with novel genetic defects.

Genetic defects were identified in 41% of all patients and in 50% (16 of 32) of the cardiologically confirmed familial forms and consisted of 1 or more mutations in 11 different genes, indicating that further studies are needed to find causes for the remaining familial forms of LVNC. Molecular diagnosis of LVNC is important because it offers reliable identification of asymptomatic relatives at risk. In the absence of an identified genetic cause for LVNC, or when relatives decline DNA testing, cardiological screening remains the appropriate method to identify familial disease.

The proportion of familial disease in this study is higher than reported previously (18% to 50%) by studies investigating the prevalence of genetic defects in adult patients or ascertaining family histories of cardiomyopathy. The systematic cardiological family screening showed that the majority (63%) of the affected relatives were asymptomatic, explaining that family histories without cardiological family studies failed to identify 44% of familial disease. Intrafamilial variability and incomplete penetrance, including asymptomatic disease, as well as small family size, may contribute to underestimation of familial disease. Therefore, cardiological evaluation of at-risk relatives of all patients with LVNC is recommended to enhance detection of familial disease, in accordance with the current expert consensus for family screening in HCM. Familial screening for cardiomyopathies is important because early diagnosis in relatives may prevent severe complications. Nevertheless, predictive DNA testing and cardiological evaluation should only take place after relatives have been well informed about possible medical benefits of early diagnosis, including suitable treatment and lifestyle recommendations as well as psychological and socioeconomic consequences of predictive testing (particularly in countries where genetic discrimination by insurance companies or employers is not prohibited).

Similar to other familial cardiomyopathies, familial LVNC showed intrafamilial phenotypic variability, including HCM and DCM and reduced penetrance (ie, clinical symptoms not expressed or expressed to a lesser degree in some persons with the familial mutation). In this study, nonpenetrance was observed in 8 relatives with a mutation ranging in age from 12 to 72 years. The risk of developing a cardiomyopathy in unaffected carriers is currently unknown and late onset cannot be excluded. Therefore, the implications of nonpenetrance include pursuing cardiological follow-up of unaffected relatives (as depicted in Figure 1). Nonpenetrance of LVNC calls for the continuation of cardiological surveillance of unaffected carriers. For families in which the genetic defect is unknown, continuation of cardiological follow-up of unaffected relatives and of family screening remains recommended until more families can be genotyped and the correct risk status of relatives can be established. Improved imaging by echocardiography and cardiac MRI has enhanced diagnosis and awareness of LVNC. However, establishing the extent to which physiological trabeculations are pathological remains difficult.

Mutations in the sarcomere genes were found in 6 of 8 affected infants tested and in 17 of 48 adult probands. Although the number of children included in this study is too small to draw conclusions on the cause of childhood disease, molecular testing of sarcomere genes and systemic cardiological evaluation of first-degree relatives are recommended in early onset LVNC, especially in absence of dysmorphic features or metabolic defects. Congenital heart malformations in patients with LVNC should not refer from analyzing sarcomere genes. Our results endorse that co-occurrence of LVNC and congenital heart defects with and without sarcomere gene
defect are not rare, warranting careful evaluation of the validity of the fourth of the Jenni diagnostic criteria.15,17,36–38

Two severely affected children and 3 adults were compound/double heterozygous, indicating that multiple mutations seem not to be significantly more prevalent in LVNC (22%) than in HCM (7%) (P = 0.15).39 In HCM, double heterozygosity for truncating sarcomere mutations have been associated with severe congenital forms of HCM, inherited in an autosomal recessive mode.40–42 In this study, double mutations also were observed in adults with LVNC. The complex genetic defects in adults involved the combination of a sarcomere gene with another gene, suggesting that 2 sarcomere mutations may cause a more severe phenotype than the combination of a sarcomere mutation and a nonsarcomere mutation. The epigenic effect of multiple mutations may depend on the specific defects involved. Further studies are needed to investigate the role of additional mutations and determine whether they play a role in the phenotypic variability.

For now, the evidence that sarcomere defects are an important cause for LVNC, together with the occurrence of LVNC, HCM, and DCM within families, suggests that these cardiomyopathies represent phenotypic variability within a spectrum and thus require comparable approach with respect to family screening.

The results of cardiological follow-up of families will help to understand the natural history of LVNC, to determine whether LVNC represents a congenital endomyocardial defect or may develop later in life, and eventually to attain recommendations for follow-up of relatives on the basis of accurate risk classification.43 The perspective of new studies investigating modifying genetic effects or genome-environment interactions to explain variability and age-dependent penetrance of this phenotype is challenging.

Acknowledgments

We are grateful to the families for their participation and to R.T. van Domburg for support in the statistical analysis.

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Disclosures

None.

References


**CLINICAL PERSPECTIVE**

In left ventricular (LV) noncompaction cardiomyopathy (LVNC), part of the LV wall is thickened and bilayered, consisting of a thick endocardial layer with prominent intertrabecular recesses and a thin, compact epicardial layer. To investigate the genetics of LVNC, we performed systematic family screening and extensive molecular testing of 58 consecutively diagnosed LVNC probands (49 adults and 9 children). This combined approach showed that 67% of LVNC is genetic in origin. In 64% of the probands, familial disease was demonstrated by screening 194 first- and second-degree relatives with electrocardiography and echocardiography. The majority (63%) of the relatives diagnosed with LVNC was asymptomatic, including 9 relatives with a mutation. Presymptomatic DNA testing also identified 8 unaffected (without the cardiac phenotype) carriers, explaining why many probands were initially unaware of familial disease. The molecular screening for mutations in 17 genes identified mutations in 11 genes in 41% of the probands (17 adults and 6 children). Most mutations were transmitted in an autosomal dominant mode. Two adults and 2 children were compound or double heterozygous for 2 different mutations. One adult proband had 3 mutations. In half of familial LVNC, the genetic defect remained inconclusive. These results show that LVNC is predominantly a genetic cardiomyopathy with variable clinical presentation ranging from asymptomatic to severe manifestations. Accordingly, the diagnosis of LVNC requires genetic counseling, DNA diagnostics, and echocardiographic family screening.
The Importance of Genetic Counseling, DNA Diagnostics, and Cardiologic Family Screening in Left Ventricular Noncompaction Cardiomyopathy

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Supplemental Material

“The Importance of Genetic Counseling, DNA Diagnostics and Cardiologic Family Screening in Left Ventricular Noncompaction Cardiomyopathy”

Supplemental Data
1. Supplemental Data
2. Supplemental Table
3. Supplemental Figures

1. Supplemental Data
Description of the family studies of LVNC probands with a gene defect

A. Adult probands
A1. Adult probands, single mutation
Cardiac β-Myosin Heavy Chain (MYH7)

Family 1
LVNC was diagnosed in a 27-year-old woman presenting with progressive dyspnea, fatigue and edema. In 1993 her sister died aged 27 years of a peripartum cardiomyopathy 6 days after giving birth to her third child.17 Cardiological screening of seven asymptomatic siblings revealed LVNC in five: two sisters aged 35 and 30 years, and two brothers aged 34 and 49 years. LVNC was also diagnosed in the 62-year-old father who was in NYHA class II and in the 24-year-old asymptomatic daughter of the eldest brother. DNA analysis showed the p.Leu301Gln mutation in the MYH7 gene in all tested affected relatives and was excluded in four asymptomatic and cardiologically unaffected relatives.

Two asymptomatic children (aged 12 and 15 years) of one of the affected relatives had LVNC, the 12-year-old girl was also diagnosed with an Ebstein malformation; both had the MYH7 mutation.

Family 2
A 35-year-old man was hospitalized with symptoms of congestive heart failure (extreme fatigue, dyspnea, orthopnea and palpitations). He was diagnosed with LVNC and severe systolic left ventricular (LV) dysfunction.17 He had a large thrombus in the left ventricle, which resolved completely with anti-coagulant treatment. Treatment of heart failure was successful and after four-and-a-half years of follow-up he remains asymptomatic with moderate LV dysfunction. In childhood, he had been treated for lymphoblastic leukemia with chemo- and radiotherapy (cytosine, arabinoside, methotrexate and prednisone). Cardiological family studies revealed LVNC in his asymptomatic son, brother, father and a paternal aunt. The medical records of a paternal uncle who died at age 70 years also indicated LVNC. All the living affected relatives had the p.Asp545Asn and p.Asp955Asn double mutation in cis in the MYH7 gene. Additionally, predictive DNA analysis found both mutations in the asymptomatic and unaffected 15-year-old daughter of the proband.
and in the unaffected son of the deceased uncle of the proband, confirming the uncle to have been a carrier of the double mutation.

Family 3
A 32-year-old woman with persistent dyspnea following an episode of influenza was diagnosed with heart failure and LVNC. She had the p.Glu1350del mutation in the \textit{MYH7} gene. Her asymptomatic mother had the \textit{MYH7} gene mutation but did not show features of LVNC, although LV function was slightly impaired (Fractional shortening 27\%) and she had a grade II aortic insufficiency. The asymptomatic father did not carry the mutation and did not show any signs of cardiomyopathy. The asymptomatic brother, an athlete, showed trabeculation of the apex and his ECG showed a nonspecific intraventricular conduction delay. Exercise ECG and 24-hrs ECG were normal. He did not have the familial mutation. His cardiac abnormalities are possibly related to athletic performance, or they may indicate involvement of another so far unidentified genetic cause for LVNC in this family.

Family 4
In 2005 LVNC and an \textit{MYH7} mutation (p.Arg1925Gly) were diagnosed in a 50-year-old asymptomatic woman. She was referred for cardiological screening after the sudden death of her brother at age 48 years due to congestive cardiomyopathy confirmed at autopsy. The \textit{MYH7} mutation was excluded in an asymptomatic and cardiologically unaffected 55-year-old sister and the asymptomatic 77-year-old mother of the patient. The father died of lung cancer at age 65 years. The family history suggested that the mutation was inherited from the paternal side because of a reported sudden death at age 64 of one of the paternal aunts. One of her sons showed apical trabeculation at age 37 years, not fulfilling the Jenni criteria and his ECG showed voltage criteria for left ventricular hypertrophy.

Family 5
Cardiac screening of an asymptomatic 22-year-old woman identified LVNC and a pathogenic \textit{MYH7} mutation (p.Asn1918Lys). Her father had died aged 59 years after developing a sepsis. He was diagnosed previously with heart failure. The father’s sister was also diagnosed with heart failure, and died at the age of 30 years. Three brothers of the father died suddenly, two at age 50 and one at age 63 years. An asymptomatic son and daughter of one of these brothers were diagnosed with LVNC at age 30 and 27 years respectively. They had the \textit{MYH7} mutation. The sole surviving paternal uncle who was diagnosed with LVNC at age 61 years also had the \textit{MYH7} mutation. The asymptomatic sister of the proband carried the \textit{MYH7} mutation, showing non-penetrance at age 27 years.

Family 6
A 41-year-old man presented with progressive dyspnea caused by congestive heart failure due to LVNC. An ICD was implanted. DNA analysis showed the p.Tyr1488Cys mutation in \textit{MYH7}. His asymptomatic 9-year-old and 12-year-old sons were screened by a pediatric cardiologist and found normal, as were his asymptomatic mother and brother.
The eldest son carried the *MYH7* mutation, the youngest didn’t. The mother and brother refused DNA testing.

**Family 7**
In 2005 a 61-year-old man had complaints of palpitations and chest pain. Echocardiography showed left ventricular hypertrophy. Two years later LVNC was diagnosed on MRI. He had the p.Leu658Val mutation in *MYH7*. Family history was negative for cardiac disease or sudden death; so far no relatives participated in cardiologic screening or DNA analysis.

**Family 8**
ECG showed left ventricular hypertrophy which could not be confirmed by echocardiography in a 21-year-old man who had a syncope while jogging. MRI at age 33 years showed evident LVNC with a non-compact to compact (NC/C) ratio of 4.2. After this diagnosis his father, previously diagnosed with HCM at age 17 years, also after syncope while jogging, had an MRI and was subsequently diagnosed with LVNC (NC/C ratio 2.2) at the age of 60 years. He died three years later. Family history reported that the probands paternal grandmother died at 40 years of age and that a sudden death during exercise occurred in a 17-year-old paternal uncle and in a 21-year-old paternal aunt. Cardiologic screening of another paternal uncle shortly before he died at age 64 years showed no abnormalities. The proband and his affected father had the p.Ile818Asn mutation in *MYH7*.

**Family 9**
Standard prenatal ultrasound at 20, 22 and 23 weeks gestation showed cardiomegaly with increased wall thickness. Advanced prenatal ultrasound at 23+3 weeks gestation showed cardiomegaly, decreased contractility and increased apical myocardial wall thickness. A fetal cardiomyopathy was suspected, prenatal follow up showed a stable condition of the pump function. Subsequent cardiologic screening identified LVNC in the 34-year-old Turkish father who suffered from fatigue and excessive perspiration. The asymptomatic Turkish mother was unaffected. Their eldest (asymptomatic) son, aged 14 years, was also unaffected; the youngest son, aged 10 years who was also asymptomatic, had LVNC. The paternal mother had been diagnosed with LVNC after having chest pain approximately one year earlier in another medical center. One of her sisters suffered a sudden cardiac death at the age of 38 years. An *MYH7* mutation was identified (c.732+1G>A) in the affected father. At gestational age 37+4 weeks their son was born. DNA analysis confirmed the presence of the *MYH7* mutation. Echocardiography showed evident LVNC with cardiomegaly. Left ventricular function was not impaired and he was discharged from the hospital one day later. Follow-up will take place at the pediatric cardiology department. The two affected sons both carried the *MYH7* mutation. The asymptomatic 28-year-old brother was unaffected, he refused DNA testing; two unaffected sisters, aged 30 and 45 years, did not carry the *MYH7* mutation.

**Myosin Binding Protein C (MYBPC3)**
Family 10
LVNC was diagnosed in a 56-year-old woman. In 1980, at the age of 30 years she was diagnosed with an unspecified cardiomyopathy. In 2000 on echocardiography a dilated cardiomyopathy was diagnosed, with a slight mitral valve insufficiency. In 2001 she suffered a mesenterial thrombosis. She was also diagnosed with myelofibrosis. She had several transient ischemic attacks and a cerebral infarction at age 53 years confirmed by MRI. DNA analysis identified the Dutch HCM founder mutation c.2373dupG in the MYBPC3 gene. She died from progression of myelofibrosis at the age of 59 years. Her father died of a peritonitis aged 87 years. At autopsy his heart showed signs of hypertrophy and dilatation of the left and right ventricle and the founder mutation was confirmed in autopsy tissue. The proband’s asymptomatic son inherited the founder mutation. He has Fallot’s tetralogy, which was surgically corrected at the age of 14 months and cardiological screening at the age of 31 years did not show features of cardiomyopathy.

**Phospholamban (PLN)**

Family 11
After successful resuscitation due to ventricular tachycardia a 48-year-old woman was diagnosed with LVNC (NC/C ratio was 2.5). She had the p.Arg14del mutation in the PLN gene. Her father died aged 62 years. Post mortem analysis showed cardiomyopathy with a thrombus in the left ventricular apex. DNA analysis of the deceased father could not be performed. Cardiologic screening of an asymptomatic 47-year-old sister, without the PLN mutation, was normal.

**Calsequestrin (CASQ2)**

Family 12
A 53-year-old man from Surinam had exercise intolerance and was scheduled for an esophagal-jejunostomy due to complications of a gastrectomy. The gastrectomy, six months previous, was due to gastric necrosis, caused by an arterial thrombo-embolism. Preoperative physical examinations revealed severe LV dysfunction due to LVNC. DNA analysis identified the p.His244Arg mutation in CASQ2. He also had the unclassified variants (UVs) p.Lys204Arg in Lim Domain-Binding 3 (LDB3) and c.12G>A in Cysteine-and-Glycine-rich protein (CSRP3). His asymptomatic mother (aged 77 years) and sister (aged 53 years) both had LVNC and the pathogenic CASQ2 mutation. The mother also had the LDB3 variant but his sister did not. The CSRP3 variant was not tested in the mother and sister.

A2. Adult probands, multiple mutations or unclassified variants

**MYBPC3 and a UV in cardiac α-Actin (ACTC1)**

Family 13
A 50-year-old man from Surinam presented with shortness of breath and palpitations, especially during stressful events. Cardiological evaluation detected non-sustained ventricular tachycardia and severe LV dysfunction. He was diagnosed with LVNC and received an ICD. DNA analysis identified the mutation p.Ala216Thr in MYBPC3 and unclassified variant *22C>T in ACTC1. Cardiologic examination of an asymptomatic daughter showed no anomalies, she did not have the MYBPC3 mutation, the ACTC1 UV has not been tested yet.
Cardiac Troponin T (TNNT2) and Lim Domain-Binding 3 (LDB3)

Family 14
In 2003 a 45-year-old woman of Moroccan descent presented with exercise related chest pain. Echocardiography showed apical hypertrophy and contrast echocardiography showed typical features of LVNC in the apex of the LV. DNA analysis identified the p. Pro87Leu mutation in TNNT2 and the p.Asp117Asn mutation in LDB3. She had six asymptomatic children who were screened cardiology. LVNC was diagnosed in two sons (aged 22 and 27 years respectively). One of these sons had the TNNT2 but not the LDB3 mutation. The other children declined DNA testing. A third son had supraventricular tachycardia and apical ventricular hypertrophy with trabeculation with a NC/C ratio <2. No signs of cardiomyopathy were detected in three children (aged 18 to 31 years).

TNNT2 and CASQ2

Family 15
A 35-year-old woman from Curacao was diagnosed with peripartum cardiomyopathy. Pregnancy was complicated by pre-eclampsia with hypertension. Echocardiography showed LVNC with an ejection fraction of approximately 20%. She had two mutations in TNNT2 (p.Arg161His and p.Val225Leu) and one in CASQ2 (p.His244Arg). So far no relatives have participated in the family study. Therefore it was not possible to determine whether the two TNNT2 mutations are allelic or not.

Lamin A/C (LMNA) and LDB3

Family 16
This patient had palpitations at age 30 and was previously diagnosed with limb girdle muscular dystrophy type IB. At age 42 years he was admitted with atrial fibrillation, non-sustained VT’s and a total AV block. Cardiac MRI diagnosed LVNC. He received an ICD. He had the c.1608+5G>C mutation in LMNA and the p.Asp117Asn mutation in LDB3. Family history was positive for dilated cardiomyopathy (two daughters of his paternal grandmother’s sister; both confirmed LMNA mutation carriers; a son and daughter of one of these daughters; a son and grandson of another sister of the paternal grandmother) and sudden cardiac death (the proband’s sister at age 39; the probands father at age 59; a paternal uncle at age 43 and a son of the first sister of the paternal grandmother at age 40 years). His asymptomatic brother had both mutations, cardiologic evaluation showed a first-degree AV-block with frequent ventricular extrasystoles and left ventricular hypertrophy on ECG. Echocardiography and MRI did not show signs of LVNC, MRI showed an ejection fraction of 47%. One of his sisters, who reported palpitations, carried only the LMNA mutation but not the LBD3 mutation. ECG showed sinus bradycardia and a first-degree AV-block. Echocardiography did not show signs of cardiomyopathy.

Tafazzin (TAZ) and an UV in LMNA

Family 17
In 2007 a 57-year-old Surinam woman was diagnosed with cardiomegaly on X-ray after referral for persisting coughing. Echocardiography showed LVNC. She had the UV c.1968+26A>G in LMNA and the p.Phe128Ser mutation in TAZ. One of her sisters was
reported with a sudden death at the age of 57 years. Her two sons were asymptomatic, one declined DNA analysis and cardiologic screening; the other was unaffected and didn’t carry either mutation. The 71-year-old sister, suffering from dyspnea and fatigue, showed significant cardiac enlargement on an X-ray made in Surinam. She carried the UV in LMNA; the results of the TAZ gene analysis are not known yet.

A3. Adult probands with unclassified variants
Three families had only unclassified variants. Although these LVNC families did not have pathogenic mutations they were added here to complete the overview of the results.

A. UV in CASQ2
Preoperative cardiologic screening revealed LVNC in a 56-year-old asymptomatic woman. Cardiologic screening identified LVNC in her asymptomatic 16-year-old son, two asymptomatic brothers, aged 54 and 57 years respectively and in the 20-year-old asymptomatic daughter of the youngest brother. In childhood she was diagnosed with an aortic coarctation that was surgically corrected at the age of four years. She also had a duplicate frontal mitral valve slip. An unclassified variant was identified in CASQ2 (p.Asp398del) in the proband. This variant did not co-segregate with LVNC in the family.

B. UV in CASQ2
This patient presented had a CVA in the right hemisphere at age 38 years. Following the young-stroke-protocol she had a cardiological examination that revealed LVNC. Her sister was diagnosed with dilated cardiomyopathy at age 21 years. She was one of the first patients in the Netherlands to receive an ICD. She died aged 23 years of complications of an ICD infection. The 66-year-old mother was diagnosed with LVNC after diagnosis was made in her eldest daughter. She had been suffering from dyspnea. Her 65-year-old asymptomatic brother and his 40-year-old asymptomatic daughter were also diagnosed with LVNC; her 55-year-old asymptomatic sister was diagnosed with DCM. Family history further mentioned a paternal nephew who has an ICD, his brother died suddenly at the age of 40 years. The UV p.Asp398del in CASQ2 has been identified in the mother of the proband but didn’t segregate with disease in the family.

C. UV in LMNA and LDB3
A 48-year-old man presented with chest pain. Catheterisation showed trabeculation and left ventricular dysfunction. LVNC was confirmed by echocardiography. During admission telemetry showed non-sustained VT’s and he received an ICD. DNA analysis showed the unclassified variants c.1968+26A>G in LMNA and p.Val118Met in LDB3. His 24-year-old daughter, who suffered from syncope, showed LV trabeculation on echocardiography. MRI confirmed LVNC. She has the LMNA UV, but not the LDB3 variant. Her 22-year-old asymptomatic sister was unaffected and carried the LMNA UV; the unaffected 53-year-old sister of the proband did not carry the LMNA UV. The LDB3 variant was not tested.

B Childhood probands
B1. Childhood probands, single mutations

**MYH7**

Family 18
A four-year-and-nine-month-old girl was diagnosed with LVNC when a cardiac murmur was detected at a pediatric consultation because of her congenital agenesis of the thyroid gland and urinary reflux. DNA analysis identified a novel nonsense mutation in the MYH7 gene (p.Tyr266X). The mutation was also identified in the 35-year-old asymptomatic father, who was subsequently screened and diagnosed with LVNC. His father had died suddenly in his sleep aged 60 years.

Family 19
A ten-year-old boy, experiencing persistent fatigue and dyspnea after having the flu, was diagnosed with heart failure and LVNC. DNA analysis revealed the p.Arg369Gln mutation in MYH7. Under medication his left ventricular function improved. Both parents and his twin sister are asymptomatic, they declined molecular and cardiologic testing.

**Cardiac α- Actin (ACTC)**

Family 20
LVNC was diagnosed in a six-week-old girl examined because of failure to thrive and feeding problems. Her heart showed mid-ventricular noncompaction with apical dilatation of the left ventricle and a dilated left atrium with bulging of the atrial septum to the right. Under treatment of an ace-inhibitor and digoxin her condition improved. DNA analysis showed a mutation in the ACTC gene, p.Met271Val. Her asymptomatic father had this mutation and was diagnosed with a dilated LV and RV, with apical, lateral and septal thickening without any evident crypts on echocardiography. On MRI LVNC was evident with a NC/C ratio of 15/3. The paternal grandparents did not have the mutation, identifying it as a de novo mutation in the father of the proband.

**α-Tropomyosin (TPM1)**

Family 21
A 16-year-old girl was suspected of liver failure. On chest X-ray she had an exceptionally large heart and echocardiography showed a poor cardiac output. She was diagnosed with LVNC. She received a left ventricular assist device before she had a heart transplant at age 17 years. DNA analysis revealed a de novo p.Arg160His mutation in TPM1: the mutation was excluded in both parents. Cardiologic screening of her mother was normal; her father had developed ischemic DCM after a major heart infarction eight years earlier, not related to the TPM1 mutation in the proband.

B2. Childhood probands, multiple mutations

**Compound heterozygosity of two mutations in MYBPC3**

Family 22
A 7-year-old girl presenting with fatigue and vomiting was diagnosed with severe dilated cardiomyopathy. X-ray and ECG showed signs of left ventricular hypertrophy. Four months after diagnosis she had a heart transplantation. The pathological exam of the heart showed a two-layered structure of the left ventricle with excessive trabeculations
consistent with LVNC. She suffered a cerebral infarction shortly after being diagnosed with LVNC. DNA analysis revealed two MYBPC3 gene mutations, the Dutch HCM founder mutation c.2373dupG and a novel mutation, p.Gly148Arg. The founder mutation was identified in the asymptomatic mother, without signs of cardiomyopathy. The maternal grandfather, diagnosed with HCM had the c.2373dupG mutation. The asymptomatic father had the p.Gly148Arg mutation and was subsequently diagnosed with LVNC. The paternal grandfather, suffered from hypertension and atherosclerosis, was diagnosed with HCM at age 76 years and had the p.Gly148Arg mutation. One asymptomatic paternal uncle (age 49 years) had the p.Gly148Arg mutation; cardiologic examination did not show signs of cardiomyopathy. The asymptomatic paternal grandmother and a paternal uncle without the mutation had no signs of cardiomyopathy. Screening of two asymptomatic brothers (ages four and nine years) of the proband did not show signs of cardiomyopathy. The eldest brother had an aortic coarctation which was surgically corrected at age 9 years (figure 3).

Double heterozygosity of mutations in cardiac Troponin I (TNNI3) and TPM1

Family 23
In 2004 a four-month-old girl was admitted because of failure to thrive, dyspnea and excessive perspiration. Echocardiography diagnosed LVNC. She suffered a cerebral infarction in the medial cerebral artery area at the age of four months. At age four years and 10 months she died waiting for a heart transplant. DNA analysis revealed the novel mutations p.Asp180Gly in the TNNI3 gene and c.241-12_241-11delinsTG in the TPM1 gene. The TPM1 mutation was detected in her asymptomatic, cardiologically unaffected father (age 39 years). Her mother (age 37 years) who was also asymptomatic and cardiologically unaffected had the mutation in the TNNI3 gene. The paternal grandparents were asymptomatic and unaffected. The paternal grandfather had the TPM1 mutation. This mutation was excluded in the paternal grandmother and an unaffected aunt. In the asymptomatic unaffected maternal grandparents, the TNNI3 mutation was identified in the grandmother and excluded in the grandfather. A maternal aunt, a professional athlete who did not have the TNNI3 mutation, had features of noncompaction of the left ventricle with a ratio less than 2 and a normal ejection fraction. It is unclear whether the attenuated form of trabeculation was primarily related to physical activity or indicates another yet unidentified genetic cause for LVNC in this family.
2. Supplemental Table

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Table 1. Overview of the family studies of 53 LVNC probands. Numbers 1-23 correspond to 1-23 and 24-26 to A-C in the addendum.
3. Supplemental Figures
Pedigrees of families with a mutation

Family 1

Family 2
MYH7 +/− p.Tyr1488Cys

Family 6

MYH7 +/− p.Leu658Val

Family 7

MYH7 +/− p.Ile818Asn

Family 8
Family 13

MYBPC3 +/-
p.Ala216Thr
ACTC +/-
*22C>T

MYBPC3 -/
ACTC -/

ND

ND

ND

ND

ND

ND

Family 14

TNNT2 +/-
p.Pro87Leu
LDB3 +/-
p.Asp117Asn / c.549-4A>G

ND

TNNT2 +/-
LDB3 ND

ND

ND

ND

ND

ND

ND
Family 17

- LMNA +/-
- TAZ +/-
- LMNA ND
- TAZ ND

Family A

- CASQ2 ND
- ND
- CASQ2 +/-
- p.Asp398del
- CASQ2 +/-
- CASQ2 ND
MYH7 +/– p.Tyr266X

Family 18

MYH7 +/–

ND

ACTC -/– p.Arg369Gln

Family 20

ACTC -/–

ND

ACTC +/– p.Met271Val

Family 21

TPM1 -/– p.Arg160His

ND

TPM1 -/–
Family 22

Family 23