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## Multigenic disease and bilineal inheritance in dilated cardiomyopathy is illustrated in non-segregating LMNA pedigrees

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### Abstract

**Background:** We have previously described 19 pedigrees with apparent lamin (*LMNA*)-related dilated cardiomyopathy (DCM) manifesting in affected family members across multiple generations. In 6 of 19 families, at least one individual with idiopathic DCM did not carry the family's *LMNA* variant. We hypothesized that additional genetic cause may underlie DCM in these families.

**Methods:** Affected family members underwent exome sequencing to identify additional genetic cause of DCM in the 6 families with non-segregating *LMNA* variants.

**Results:** In 5 of 6 pedigrees, we identified at least one additional rare variant in a known DCM gene that could plausibly contribute to disease in the *LMNA* variant-negative individuals. Bilineal inheritance was clear or presumed to be present in 3 of 5 families and was possible in the remaining two. At least one individual with a *LMNA* variant also carried a variant in an additional identified DCM gene in each family. Using a multivariate linear mixed model for quantitative traits, we demonstrated that the presence of these additional variants was associated with a more severe phenotype after adjusting for sex, age, and the presence/absence of the family's non-segregating *LMNA* variant.

**Conclusions:** Our data support DCM as a genetically heterogeneous disease with, at times, multigene causation. Although the frequency of DCM resulting from multigenic cause is uncertain, our data suggest it may be higher than previously anticipated.

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## Keywords

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## Introduction:

Recent advances in next-generation sequencing have facilitated significant progress in identification of putative causative variants in dilated cardiomyopathy (DCM) genes<sup>1</sup>. Nevertheless, a comprehensive understanding of the genetic architecture of idiopathic DCM (IDC) remains elusive. It has been previously estimated that 20–35% of DCM can be demonstrated to be familial when clinical screening of at-risk relatives of an IDC proband is conducted<sup>2–5</sup>. In many of these cases, DCM presents as an adult-onset, autosomal dominant, Mendelian condition with reduced, age-dependent penetrance and variable expressivity. Our hypothesis has been that DCM, regardless of familial occurrence, has a predominantly genetic basis<sup>6, 7</sup>.

Because most DCM manifests in adulthood, it is not unusual to identify pedigrees with multiple affected individuals who carry the family mutation but who have not yet shown any clinical (phenotypic) evidence of disease. These mutation-positive, phenotype-negative individuals are consistent with the well-known age-dependent penetrance of DCM. In contrast, individuals with IDC who do not carry the family's pathogenic variant are more atypical. The traditional explanation has been that these individuals represent “phenocopies” – that is, individuals whose DCM stems from non-Mendelian causes such as long-standing hypertension, environmental exposure, or other unknown factors.

Mendelian models of genetic disease are premised on the idea that a single locus is responsible for disease in all affected members of an identified family. In these models, pedigree-based genetic testing aims to identify high-risk variant alleles that clearly segregate with disease and are exceedingly rare or absent from unaffected relatives and control populations. Not all observed segregation patterns conform to Mendelian expectations, however, and recent years have seen the emergence of multigene models for a diverse array of disorders<sup>8–11</sup>, including genetic cardiovascular disease<sup>12–15</sup>. Based on these models and unpublished data from our own family studies we have developed the working hypothesis that a proportion of familial DCM may similarly be explained by combined effect of multiple genetic loci. This focus remains novel for DCM genetics.

Sequence variants in the lamin A/C (*LMNA*) gene represent one of the most common genetic causes of DCM<sup>16</sup>. As conduction system disease (CSD) typically precedes onset of cardiomyopathy by a median of seven years<sup>17</sup>, detection of a clinically actionable *LMNA* variant can guide management towards detection and treatment of arrhythmia, as well as genetic screening of additional family members at risk for CSD and DCM. In 2008, in an effort to determine the frequency of rare *LMNA* variants among individuals with IDC, we conducted Sanger sequencing of a cohort of 324 unrelated patients with clinically confirmed IDC<sup>18</sup>, defined as left ventricular enlargement (LVE) with systolic dysfunction occurring in the absence of other clinically detectable causes<sup>19</sup>. We identified 19 pedigrees carrying 18

unique, protein-altering *LMNA* variants, representing an overall yield of 5.8% for the cohort. Unexpectedly, in 6 of the 19 families, one or more individuals with IDC did *not* carry the familial *LMNA* variant. At the time of publication, we defined these as “non-segregation” pedigrees and speculated that additional, presumably unlinked, genetic cause(s) may contribute to the DCM in these families.

We herein summarize follow-up genetic studies in these families. Our analyses have expanded some of the originally published pedigrees and include a more informative approach to DCM phenotyping. In 5 of 6 pedigrees (Figures 1, 2, 3, 4, 5), additional genetic information now provides evidence for at least one other rare variant contributing to DCM.

## Methods:

All study methods are available as Supplemental Materials. For purposes of reproduction and replicability, the data and programs used for statistical analyses are publicly available at [https://github.com/kinnamon-lab/lmna\\_nonseg](https://github.com/kinnamon-lab/lmna_nonseg). Informed consent was obtained from all study participants following approval by the Oregon Health & Science University, the University of Miami, or the Ohio State University.

## Results:

The overall clinical profile of our DCM cohort has already been extensively described<sup>19</sup>. Prior sequencing of a subset of 312 unrelated probands identified *LMNA* variants in 19 pedigrees with evidence of familial disease<sup>18</sup>. In 6 families, the identified *LMNA* variant did not completely segregate with observed disease phenotypes suggesting existence of additional causative genetic factors. The majority of these pedigrees have been expanded with additional clinical, family, or genetic data since their initial publication<sup>1, 18, 20–22</sup> and are presented in updated form in Figures 1–5. For consistency, these pedigrees retain their previously published letter designations<sup>18–20</sup>. In order to systematically analyze potential genotype/phenotype correlations, affection status for each individual with DCM was categorized using an ordinal grading scale ranging from 0 (no phenotypic evidence of DCM) to 4 (DCM with advanced disease) (Table 1). These grades were then applied to the pedigrees.

Exome sequences were obtained for a total of 20 individuals across all six pedigrees (indicated by an “E”). Only one pedigree (Pedigree B, not shown) was uninformative as genetic cause remained unresolved in 2 affected individuals lacking the familial *LMNA* mutation. This pedigree is not discussed further. In the remaining 5 families, all of which were Non-Latino Caucasian, sequencing identified at least one other rare genetic variant affecting another plausible disease locus (Table 2). For each pedigree, these additional variants met many of the usual criteria for potential disease-association (rare, occurred in conserved regions of genes with strong biological associations with DCM), but did not meet ACMG criteria for pathogenicity or likely pathogenicity (Suppl. Table 1). Bilineal inheritance could be clearly determined or inferred from 2 pedigrees (O, S). Based on available evidence, bilineal inheritance was presumed in one other family (P), and possible in two others (L, N).

## Pedigree L:

In addition to a previously reported p.(Ala318Thr) *LMNA* variant<sup>18</sup>, exome sequencing identified rare missense variants affecting *BAG3*, p.(Arg71Trp)<sup>1</sup>; *RBM20*, p.(Met338Thr); and *DSP*, p.(Arg2826His); as well as a truncating, A-band *TTNiv* variant (*TTNiv*), p.(Pro24523Hisfs\*4) in the proband (L.3) and his mother (L.2), who did not carry the *LMNA* variant (Pedigree L, Figure 1). Although variants in *BAG3* are well known to cause advanced disease, with less evidence for missense *DSP* variants, predictive analyses were divided for the *BAG3* and *DSP* variants, with half of the tested algorithms supporting pathogenicity of each allele. The p.(Pro24523Hisfs\*4) *TTNiv*, which was predicted to affect the A-band and severely truncate the full-length protein (24525 vs 35991 amino acids), was carried by both affected family members. While absent from online databases of genetic variation, the *RBM20* variant occurred outside of the exon 9 hotspot and was predicted to be benign by almost all tested predictive pathogenicity algorithms (Suppl. Table 2). While both L.2 and L.3 exhibited severe (Grade III/IV) DCM and CSD, onset occurred two decades earlier in the proband than in his mother, suggesting potential for increased disease severity in the presence of the *LMNA* variant. As is true for all other variants identified in this study (Table 2, Suppl. Table 2), the 5 variants identified in this family are considered to be VUSs under current ACMG guidelines.

## Pedigree N:

A rare p.(Arg716Gln) *RBM20* variant was identified in 18 members of a large family with DCM and CSD (Pedigree N, Figure 2). This family, which also carries an p.(Arg388His) *LMNA* variant, has previously been described as pedigree F in Li et al.<sup>21</sup> and is presented here in updated form. Both parents (N.1 and N.2) of the affected proband (N.8) survived into their 70s with reported histories of heart failure and cardiomyopathy, suggesting potential for bilineal inheritance. Five of the proband's 6 siblings (N.4, N.5, N.9, N.13, N.15) developed Grade III/Grade IV DCM by the third to fifth decade. A total of 5 individuals in the second and third generation could be definitively classified as carriers of both *RBM20* and *LMNA* variants, either through direct DNA testing (N.8, N.15, N.23, N.25) or inferred from pedigree structure (N.9). Three double carriers in the second generation exhibited severe (Grade III/IV) disease (N.8, N.9, N.15), while the youngest (N.23, N.25) lacked either clinical or subclinical signs of DCM at time of screening. One *RBM20* single variant carrier (N.27) had clinically detectable DCM by 33 years of age, while 7 additional members of the 2 youngest generations exhibited signs of subclinical disease (Grade II)(N. 35) or early signs of LVE and/or systolic dysfunction not meeting clinical thresholds for DCM (Grade I) (N.19, N.22, N.28, N.29, N.30, N.31). One other family member in the third generation carried the *RBM20* variant but was phenotypically unaffected (N.26). The average age of onset for DCM in this family was 37.6 years, older than the average age of non-penetrant *RBM20* variant carriers (23.5 years), supporting possible age-related non-penetrance in affected family members. Three subjects carried only the *LMNA* variant (N. 33, N.34, N.36) and all were similarly unaffected. Two additional family members (N.4, N. 13) had unknown *LMNA* variant status, but exhibited Grade IV disease. While N.4 could be definitively determined to carry the *RBM20* variant, the mutation status of N.13 is unknown. On a population level, both the *RBM20* and *LMNA* variants are extremely rare and impact

highly conserved residues (Suppl. Table 2). Supporting pathogenicity, nuclear morphology and lamin A subcellular localization were markedly affected by the p.(Arg388His) *LMNA* variant with most affected protein aberrantly localizing to the cytoplasm<sup>20</sup>. Collectively these data support potential contribution of both variants to DCM in this family. As a single severely affected individual in the second generation (N.5) as well as two individuals in the youngest generation with early evidence of DCM not meeting formal criteria (N.22, N.28) tested negative for both variants, future genetic testing efforts may help to elucidate whether any additional genetic variation is relevant to this family.

### Pedigree O:

A rare p.(Arg399Cys) *LMNA* variant was identified in the proband (O.11) of Pedigree O (Figure 3), but not in his affected father (O.8). The proband, his mother (O.9), and his maternal grandfather (O.3) all carried the *LMNA* variant, with evidence of mild *LMNA*-related cardiac phenotype in O.3 and his brother, the proband's maternal great uncle (O.5). The proband had no known antecedent CSD or arrhythmias consistent with *LMNA*-related cardiomyopathy, but rather presented with advanced heart failure with extremely severe DCM requiring rescue ventricular assist device (VAD) placement and transplantation by 15 years of age. Exome sequencing subsequently identified a paternally inherited p.(Asp24224Ilefs\*8) *TTM* variant transmitted to both the proband and his unaffected brother (O.10) through the father (O.8) from the paternal grandmother (O.2). As with the p.(Pro24523Hisfs\*4) *TTM* variant identified in pedigree L, the p.(Asp24224Ilefs\*8) *TTM* variant affected the A-band and was predicted to severely truncate the full-length TTN protein (24230 vs 35991 amino acids). The proband's father died at 45 years following transplantation for severe DCM and heart failure that presented at 36 years. Remarkably, the paternal grandparents (O.1, O.2), also had Class III disease, and might have contributed not only the *TTM* variant (from O.2) but also a second unknown risk allele (from O.1) to cause the early onset of DCM in O.8. It is possible that co-occurrence of the paternal *TTM* variant and maternal *LMNA* variant in the proband contributed to his very severe and early onset disease. Supporting this interpretation were damaging predictions from most predictive algorithms for the *LMNA* variant, although no obvious cellular effect was indicated by nuclear imaging studies<sup>20</sup>. Notably, both variants affected conserved residues and were extremely rare (*LMNA*) or absent (*TTN*) from control populations (Table 2).

### Pedigree P:

A C-terminal paternally inherited p.(Ser437Hisfs\*2) *LMNA* variant was identified in 7 individuals (P.1, P.3, P.8, P.10, P.14, P.16, P.20) spanning 3 generations of a large family with multigenerational DCM and arrhythmia (Pedigree P, Figure 4). In 6 of 7 *LMNA* variant carriers, cardiomyopathy manifested as subclinical symptoms or early signs not meeting formal diagnostic criteria. Nevertheless, this variant was predicted to prematurely truncate the encoded lamin A and C proteins (by 227 and 135 amino acids, respectively) and highly abnormal lamin A aggregation in nuclear imaging studies strongly supported functional effect<sup>20</sup>. Exome sequencing and follow-up targeted testing subsequently identified a rare p.(Glu485Lys) *NEXN* variant in 4 family members (P.11, P.18, P.20, P.23). The proband (P.18) and her daughter (P.23) carried both the *LMNA* and *NEXN* variants and each exhibited

severe (Grade IV) DCM, either requiring transplantation (P.18) or resulting in mortality (P.23). The proband's sister (P.20), the only other known double variant carrier, was less severely affected. Their mother (P.11), the sole known family member to carry only the variant *NEXN* allele, showed no evidence of cardiovascular disease. What remains unexplained is the even earlier onset of disease and mortality from advanced heart failure in P.23, who also carried both variants. While we are at this time unable to assign a specific cause, it is possible that P.23 may have had a *de novo* third modifier, or may have inherited a possible third modifier through P.17, whose personal and family history is unknown. This postulate is suggested by Grade II DCM in P.22, who carried neither the *LMNA* nor the *NEXN* variants and whose disease was identified at 27 years by cascade clinical screening following his sister's diagnosis. Such an early age of onset is highly suggestive of a genetic cause.

### Pedigree S:

We identified a rare p.(Arg25Cys) *PLN* variant, shown to disrupt PLN function,<sup>34</sup> in multiple individuals spanning 2 generations of a severely affected family that also carried a paternal p.(Arg654\*) *LMNA* nonsense variant (Pedigree S, Figure 5). The presence of severe disease on both sides of the family as well as in single variant carriers was highly suggestive of bilineal inheritance of 2 pathogenic alleles. The p.(Arg25Cys) *PLN* variant was identified in the proband (S.9), as well as in 2 of 3 sisters (S.8, S.10) and his affected mother (S.7). All 4 carriers developed arrhythmia requiring ICD placement alongside severe (Grade IV) adult-onset DCM progressing to heart failure. Both the youngest sister (S.11) and her paternal uncle (S.5) were also severely affected but carried only the *LMNA* variant. An p.(Asp103Val) *MYPN* variant was additionally identified in S.5, but was not identified in any other family member. Usual criteria for variant adjudication (amino acid conservation, pathogenic potential by predictive algorithms, absence from control populations) supported pathology of both the *PLN* and *LMNA* variants (Table 2, Suppl. Table 2) while *in vitro* lamin A nuclear imaging provided additional evidence for pathologic *LMNA* variant effect<sup>20</sup>. A single subject, S.9 (the proband), carried both variants. While degree of DCM was generally comparable between the proband and his siblings (S.8 to S.11), there was an increased burden of arrhythmias, including sudden cardiac death and atrial fibrillation. Disease onset was not significantly earlier than in single variant carriers.

### Modeling the Impact of Non-*LMNA* Variants on Disease Severity:

We used a linear mixed model (see Methods in Suppl. Material) to determine whether the presence of additional potentially relevant variants besides the family's *LMNA* variant was associated with DCM-relevant quantitative traits. Table 3 presents descriptive statistics for the individuals in the pedigrees in Figures 1–5, and the mixed model results are presented in Table 4. The variance components point estimates are reasonable and reflect the expected negative correlation between LVEF and LVEDD z-score and phenotypic grade as well as the positive correlation between LVEDD z-score and phenotypic grade. The imprecision in these estimates precludes further inference about the additive contribution of unmeasured loci to phenotypic variance. The mean model intercept parameters estimate the mean trait values for males not carrying any of the family's variants at the average evaluation age. Trait values did



not differ substantially for females, although each increase of 1 standard deviation in the age was associated with a decrease in LVEF and an increase in phenotypic grade. The presence of the familial *LMNA* variant itself had an equivocal effect on phenotype, likely reflecting the lack of segregation in these families. However, the presence of one or more of the family's non-*LMNA* variants was associated with a measurably worse phenotype, including a decrease of 12.6% (95% CI: -23.0 to -2.3) in LVEF and an increase of 1.44 (95% CI: 0.19 to 2.68) in LVEDD z-score. While we cannot formally exclude an effect on phenotypic grade of zero, the increase of 0.8 (95% CI: -0.1 to 1.7) is also concordant with the results for the other traits. Conclusions regarding the effects of *LMNA* and non-*LMNA* variants were unchanged in models that (1) included LVEF and LVEDD z-score only and (2) modeled the effect of the number of the family's non-*LMNA* variants rather than simply presence/absence (data available on GitHub at [https://github.com/kinnamon-lab/lmna\\_nonseg](https://github.com/kinnamon-lab/lmna_nonseg)).

We used causal diagrams<sup>26-29</sup> to facilitate interpretation of genetic effects in the linear mixed model and determine whether the set of adjustment variables was appropriate (Methods and Suppl. Figure 1). Under the assumed causal model, the genetic effects in the linear mixed model can be interpreted as total causal effects of an increased burden of putatively pathogenic rare variants on the quantitative trait under typical diagnostic and treatment protocols. As such, they are indicative of genetic effects on the quantitative trait not counteracted by typical treatment protocols. Importantly, adjustment for treatment history at the time of data collection in the linear mixed model is inappropriate because it would render the genetic effects biased estimates of both total causal effects and direct causal effects with treatment held constant. On the basis of this result, we did not perform any analysis adjusting for treatment history.

## Discussion:

Our observation of variant non-segregation in 6 of 19 families with *LMNA*-related DCM was originally met with skepticism<sup>18</sup>. The current work now presents variants putatively relevant for DCM that explain additional phenotypic variation in 5 of these 6 pedigrees. This strengthens our sense that the traditional view of IDC as a strictly monogenic disorder may be incomplete<sup>6</sup>.

Non-*LMNA* variants identified in our study affected genes with known DCM associations. *RBM20* variants are estimated to occur in approximately 2% of all patients with IDC (Suppl. Table 1). The p.(Arg716Gln) variant detected in Pedigree N was 1 of 6 unique variants previously reported in our wider DCM cohort<sup>21</sup> and, like the newly identified p.(Met338Thr) variant detected in pedigree L, affected residues outside of the exon 9 hotspot<sup>30</sup>. *RBM20* encodes an RNA splicing protein that regulates splicing of several other heart-relevant genes, including titin (*TTN*)<sup>31</sup>. We detected one *TTN*v, p.(Pro24523Hisfs\*4), in Pedigree L and another, p.(Asp24224Ilefs\*8), in Pedigree O. Both variants were predicted to prematurely truncate the large cardiac N2BA transcript at the highly conserved A-band. Rare variants were additionally identified in 5 other DCM associated genes: *BAG3*<sup>l</sup>, encoding a Bcl-2-associated molecular chaperone with roles in Z-disc assembly and stress-induced apoptosis; *DSP*<sup>24</sup>, encoding a key component of desmosomal junctions in the heart;

*MYPN*<sup>32</sup>, encoding a z-disc protein with roles in sarcomere assembly and cardiac gene expression, *NEXN*<sup>33</sup>, encoding an f-actin binding protein important for maintenance of the sarcomere z-line; and *PLN*<sup>34</sup>, encoding a key regulator of calcium homeostasis and myocardial contractility.

Our multivariate linear mixed model demonstrated that the presence of one or more non-*LMNA* variants was associated with a measurably worse phenotype, including a decrease of 12.6% (95% CI: -23.0 to -2.3) in LVEF and an increase of 1.44 (95% CI: 0.19 to 2.68) in LVEDD z-score, after adjusting for sex, age, and the presence/absence of the family's non-segregating *LMNA* variant. The adjusted impact of each family's *LMNA* variant on these traits was equivocal in our model, although this result could arise if causal *LMNA* variants have more subtle effects on these quantitative traits that were not captured with adequate precision in our modest sample. The availability of *in vitro* evidence supporting pathogenicity for some of these variants<sup>20</sup> buttresses this interpretation, but statistical confirmation would require a larger sample of extended pedigrees.

In our cardiovascular genetics practice we are careful to avoid stating that lack of an identified family variant, even one considered pathogenic, relieves family members who do not carry that variant (or variants) of any future disease liability. We rather state that the risk is decreased, as other genetic and/or environmental risk factors<sup>35</sup> may be present. While useful as a framework for variant adjudication in classic Mendelian pedigrees, current ACMG guidelines<sup>23</sup> do not allow for sufficient flexibility to accurately assess contributions of multiple putative disease variants. This is particularly true when detected variants impact known disease genes that otherwise meet criteria for plausible pathogenicity. For the purposes of this study, we have considered rare variants that may be classified as VUSs to be of similar interest as those classified as pathogenic or likely pathogenic using existing ACMG criteria. Our statistical model suggests that these variants may in fact be relevant and may even explain more of the variation in phenotypic severity than the more established *LMNA* variants in these pedigrees. Future analyses will benefit greatly from increased knowledge of influencing environmental factors, protective variants, regulatory elements, or other systemic factors. In addition, continued cataloguing of normal human variation in online genetic databases (ExAC, gnomAD) and careful phenotyping of increasing numbers of patients will help to further refine determinations of variant expressivity and penetrance.

### Study Limitations:

By definition, exome-based sequencing strategies preclude detection of structural variants and other relevant variants affecting regulatory or functionally significant non-coding domains. Second, while pedigrees have been expanded to incorporate all available clinical and genetic information, small sizes or unavailability of genetically relevant subjects in some families limited our ability to more fully assess possible genotype/phenotype correlations. Third, while we have already emphasized the limited applicability of current ACMG variant adjudication guidelines to multi-variant models, all variants identified in this study must be considered to be VUSs in the absence of additional supportive genetic or functional data. Adjudications may change with access to much larger datasets. Even larger exome datasets may result in future reassessment or provide new, previously unforeseen



insights into variant pathogenicity. The multivariate linear mixed model used in our analysis is at best an approximation of a complex biological system, and simplifying assumptions were required to make the model tractable with our modest sample size. Phenotypic grade was modeled as a quantitative trait despite being defined on an ordinal scale. Goodness of fit tests showed no evidence of lack of fit, but these tests have extremely limited power with the number of pedigrees analyzed. Moreover, variance components were estimated rather imprecisely so conclusions about additive genetic variance and covariance from unmeasured loci could be drawn. A significantly larger sample of pedigrees would remedy this issue. Finally, the type of complete-case analysis performed by Mendel is valid if an individual's probability of having missing data is independent of his or her phenotype values given his or her predictors.<sup>36, 37</sup> Although this assumption cannot be empirically tested, it is reasonable as long as (1) phenotype values do not directly affect the probability of being a complete case and (2) measured genotypes, sex, and age are the only factors affecting phenotype values that also affect this probability.

## Conclusions and Summary:

In summary, we have identified additional putative genetic contributors to DCM in 5 of 6 families with non-segregating *LMNA* variants, 3 with clear evidence of bilineal inheritance. We suggest that this yield (5 of the 19 families, 26%) places multigene causation as a significant and plausible alternative to the more usual single gene Mendelian DCM model in some cases. Our data suggest that the extent of DCM arising from multigenic cause may be higher than previously anticipated. Much larger ongoing<sup>7</sup> and future studies will be required to more precisely determine the genetic architecture of DCM.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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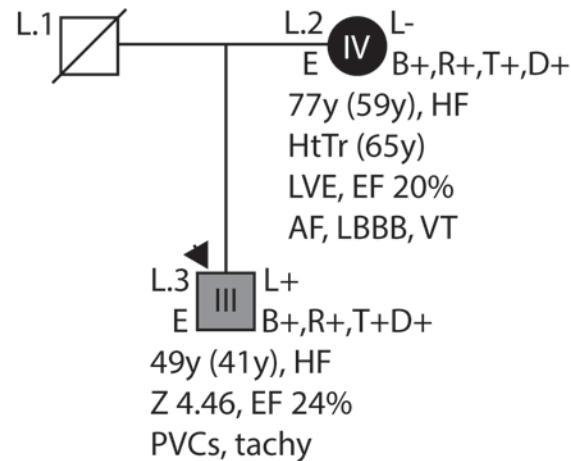
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## Pedigree L - LMNA (L), BAG3 (B), RBM20 (R), TTN (T), DSP (D)



## Severity

		Grade IV	Advanced DCM requiring interventions or DCM-related death
		Grade III	Symptomatic DCM, treatable and stable with or without medical therapy
		Grade II	Asymptomatic DCM (identified serendipitously, including screening)
		Grade I	Early evidence of DCM but not meeting formal DCM diagnosis
		Grade 0	No CV disease or CV abnormalities of uncertain relationship to DCM

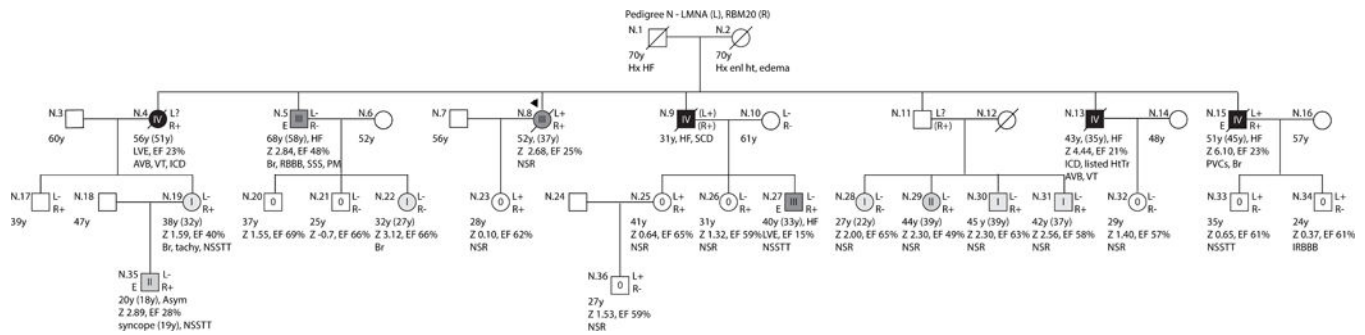
◀ Proband identifier

Patient LMNA mutation status  
 Exome 2nd locus mutation status  
 Clinical details

**Figure 1: Pedigrees with non-segregating LMNA variants, Pedigree L.**

As per convention, the proband for each family is indicated by an arrow. Males are shown as squares and females as circles. Deceased individuals are denoted by a diagonal slash. Individuals for whom exome sequencing was completed are marked on the left side with an E. Genetic testing results are indicated on the right side by either a + (variant present) or a – (variant absent). Gene abbreviations are provided in the title of each pedigree. Obligate carriers are marked in parentheses (+). The upper right symbol reports genetic testing results for *LMNA*, while the bottom right symbols report testing results for additional identified loci (Table 2). Clinical data is structured as previously described<sup>19</sup>. First line: current age if the individual is still living or age of death if the individual is deceased, age of onset (in parentheses), major diagnosis or cause of death. Second line: age of transplantation, if applicable. Third line: left ventricular size defined by magnitude of standard deviation (Z-score, z) and function defined by left ventricular ejection fraction (EF, %). Fourth line:

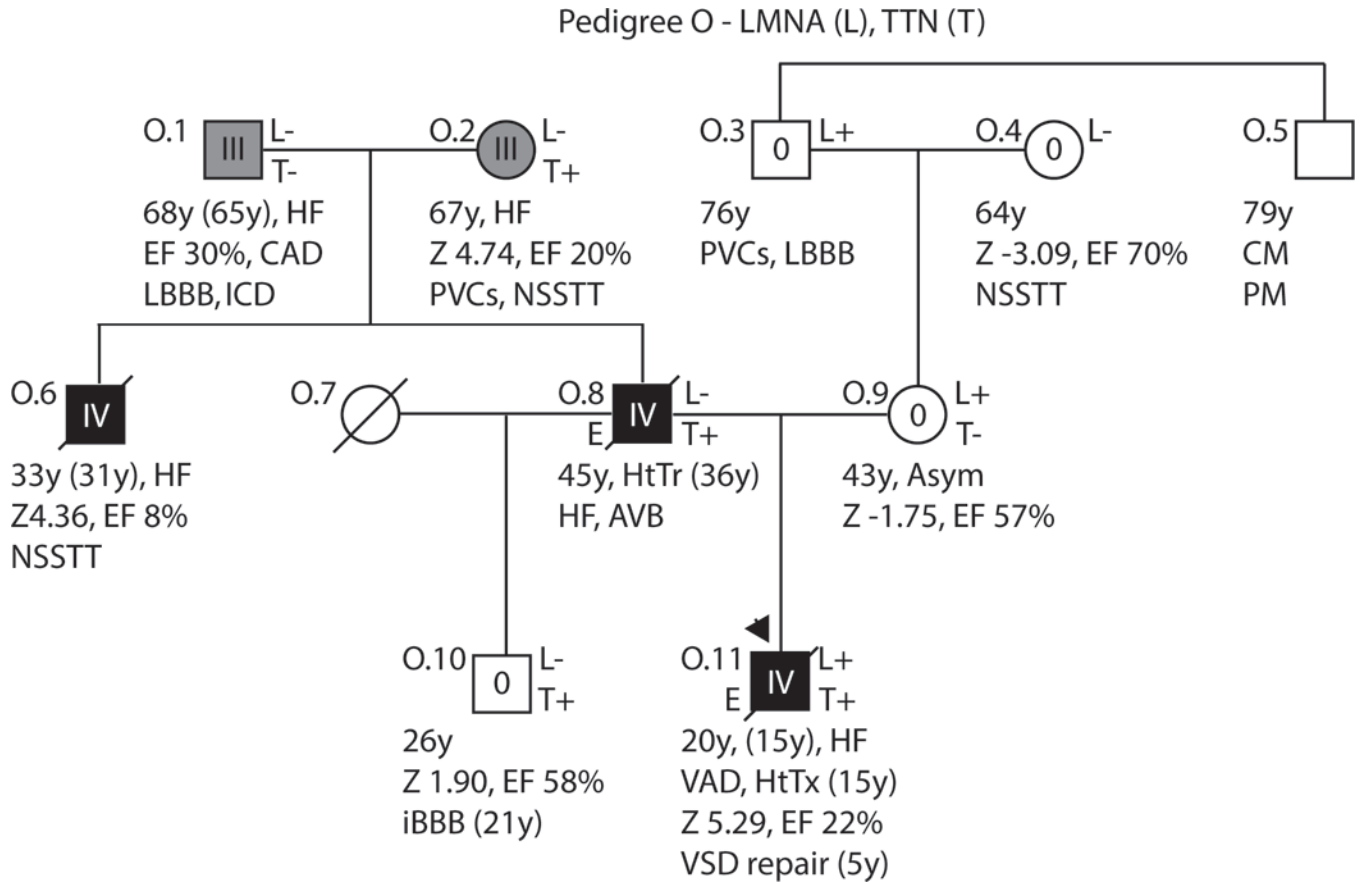
arrhythmias or other conduction system disease. Abbreviations: *AFib*, atrial fibrillation; *Asym*, asymptomatic; *AVB* atrioventricular block; *1AVB* or *2AVB*, first-degree or second-degree AVB, respectively; *A/W*, alive and well; *BiFB*, bifascicular block; *Br*, bradycardia; *CAD*, coronary artery disease; *CHD*, congenital heart disease; *CM*, cardiomyopathy, unknown type; *CVD*, cardiovascular disease; *DCM*, dilated cardiomyopathy; *EF*, ejection fraction; *HF*, heart failure; *HTN*, hypertension; *HtTr*, heart transplant; *ICD*, implantable cardioverter defibrillator; *IDC*, idiopathic dilated cardiomyopathy; *IBBB*, incomplete bundle branch block; *IRBBB* or *ILBBB*, right or left IBBB, respectively; *IVCD*, interventricular conduction delay; *LBBB*, left bundle branch block; *MVP*, mitral valve prolapse; *NSR*, normal sinus rhythm; *NSSTT*, nonspecific ST-T changes on ECG; *NSVT*, non-sustained ventricular tachycardia; *PACs*, paroxysmal atrial contractions; *PM*, pacemaker; *PSVT*, paroxysmal supraventricular tachycardia; *PVCs*, premature ventricular contractions; *RBBB*, right bundle branch block; *SCD*, sudden cardiac death; *tachy*, sinus tachycardia; *SSS*, sick sinus syndrome; *VSD*, ventricular septal defect; *VT*, ventricular tachycardia; *Z*, Z-score of left ventricular end-diastolic dimension.



**Figure 2: Pedigrees with non-segregating LMNA variants, Pedigree N.**

As per convention, the proband for each family is indicated by an arrow. Males are shown as squares and females as circles. Deceased individuals are denoted by a diagonal slash. Individuals for whom exome sequencing was completed are marked on the left side with an E. Genetic testing results are indicated on the right side by either a + (variant present) or a – (variant absent). Gene abbreviations are provided in the title of each pedigree. Obligate carriers are marked in parentheses (+). The upper right symbol reports genetic testing results for *LMNA*, while the bottom right symbols report testing results for additional identified loci (Table 2). Clinical data is structured as previously described<sup>19</sup>. First line: current age if the individual is still living or age of death if the individual is deceased, age of onset (in parentheses), major diagnosis or cause of death. Second line: age of transplantation, if applicable. Third line: left ventricular size defined by magnitude of standard deviation (*Z*-score, *z*) and function defined by left ventricular ejection fraction (EF, %). Fourth line: arrhythmias or other conduction system disease. Abbreviations: *AFib*, atrial fibrillation; *Asym*, asymptomatic; *AVB* atrioventricular block; *I**AVB* or *2**AVB*, first-degree or second-degree AVB, respectively; *A/W*, alive and well; *BiFB*, bifascicular block; *Br*, bradycardia; *CAD*, coronary artery disease; *CHD*, congenital heart disease; *CM*, cardiomyopathy, unknown type; *CVD*, cardiovascular disease; *DCM*, dilated cardiomyopathy; *EF*, ejection fraction; *HF*, heart failure; *HTN*, hypertension; *HtTr*, heart transplant; *ICD*, implantable cardioverter defibrillator; *IDC*, idiopathic dilated cardiomyopathy; *IBBB*, incomplete bundle branch block; *IRBBB* or *ILBBB*, right or left IBBB, respectively; *IVCD*, interventricular conduction delay; *LBBB*, left bundle branch block; *MVP*, mitral valve prolapse; *NSR*, normal sinus rhythm; *NSSTT*, nonspecific ST-T changes on ECG; *NSVT*, non-sustained ventricular tachycardia; *PACs*, paroxysmal atrial contractions; *PM*, pacemaker; *PSVT*, paroxysmal supraventricular tachycardia; *PVCs*, premature ventricular contractions; *RBBB*, right bundle branch block; *SCD*, sudden cardiac death; *tachy*, sinus tachycardia; *SSS*, sick sinus syndrome; *VSD*, ventricular septal defect; *VT*, ventricular tachycardia; *Z*, *Z*-score of left ventricular end-diastolic dimension.





**Figure 3: Pedigrees with non-segregating *LMNA* variants, Pedigree O.**

As per convention, the proband for each family is indicated by an arrow. Males are shown as squares and females as circles. Deceased individuals are denoted by a diagonal slash. Individuals for whom exome sequencing was completed are marked on the left side with an E. Genetic testing results are indicated on the right side by either a + (variant present) or a – (variant absent). Gene abbreviations are provided in the title of each pedigree. Obligate carriers are marked in parentheses (+). The upper right symbol reports genetic testing results for *LMNA*, while the bottom right symbols report testing results for additional identified loci (Table 2). Clinical data is structured as previously described<sup>19</sup>. First line: current age if the individual is still living or age of death if the individual is deceased, age of onset (in parentheses), major diagnosis or cause of death. Second line: age of transplantation, if applicable. Third line: left ventricular size defined by magnitude of standard deviation (Z-score, z) and function defined by left ventricular ejection fraction (EF, %). Fourth line: arrhythmias or other conduction system disease. Abbreviations: *AFib*, atrial fibrillation; *Asym*, asymptomatic; *AVB* atrioventricular block; *1AVB* or *2AVB*, first-degree or second-degree AVB, respectively; *A/W*, alive and well; *BiFB*, bifascicular block; *Br*, bradycardia; *CAD*, coronary artery disease; *CHD*, congenital heart disease; *CM*, cardiomyopathy, unknown type; *CVD*, cardiovascular disease; *DCM*, dilated cardiomyopathy; *EF*, ejection fraction; *HF*, heart failure; *HTN*, hypertension; *HtTr*, heart transplant; *ICD*, implantable cardioverter defibrillator; *IDC*, idiopathic dilated cardiomyopathy; *IBBB*, incomplete bundle branch block; *IRBBB* or *ILBBB*, right or left IBBB, respectively; *IVCD*, interventricular

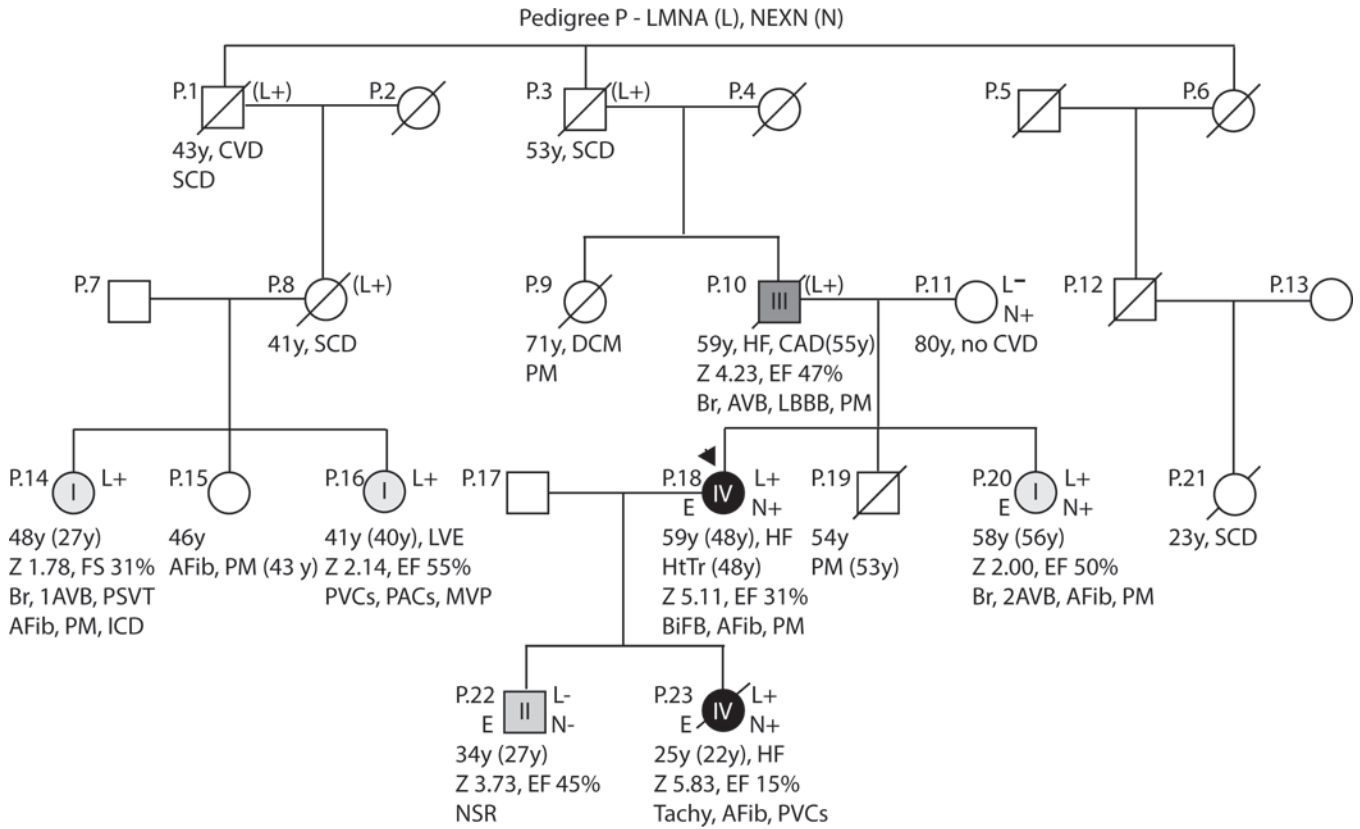
conduction delay; *LBBB*, left bundle branch block; *MVP*, mitral valve prolapse; *NSR*, normal sinus rhythm; *NSSTT*, nonspecific ST-T changes on ECG; *NSVT*, non-sustained ventricular tachycardia; *PACs*, paroxysmal atrial contractions; *PM*, pacemaker; *PSVT*, paroxysmal supraventricular tachycardia; *PVCs*, premature ventricular contractions; *RBBB*, right bundle branch block; *SCD*, sudden cardiac death; *tachy*, sinus tachycardia; *SSS*, sick sinus syndrome; *VSD*, ventricular septal defect; *VT*, ventricular tachycardia; *Z*, Z-score of left ventricular end-diastolic dimension.

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**Figure 4: Pedigrees with non-segregating LMNA variants, Pedigree P.**

As per convention, the proband for each family is indicated by an arrow. Males are shown as squares and females as circles. Deceased individuals are denoted by a diagonal slash. Individuals for whom exome sequencing was completed are marked on the left side with an E. Genetic testing results are indicated on the right side by either a + (variant present) or a - (variant absent). Gene abbreviations are provided in the title of each pedigree. Obligate carriers are marked in parentheses (+). The upper right symbol reports genetic testing results for LMNA, while the bottom right symbols report testing results for additional identified loci (Table 2). Clinical data is structured as previously described<sup>19</sup>. First line: current age if the individual is still living or age of death if the individual is deceased, age of onset (in parentheses), major diagnosis or cause of death. Second line: age of transplantation, if applicable. Third line: left ventricular size defined by magnitude of standard deviation (Z-score, z) and function defined by left ventricular ejection fraction (EF, %). Fourth line: arrhythmias or other conduction system disease. Abbreviations: *AFib*, atrial fibrillation; *Asym*, asymptomatic; *AVB* atrioventricular block; *1AVB* or *2AVB*, first-degree or second-degree AVB, respectively; *A/W*, alive and well; *BiFB*, bifascicular block; *Br*, bradycardia; *CAD*, coronary artery disease; *CHD*, congenital heart disease; *CM*, cardiomyopathy, unknown type; *CVD*, cardiovascular disease; *DCM*, dilated cardiomyopathy; *EF*, ejection fraction; *HF*, heart failure; *HTN*, hypertension; *HtTr*, heart transplant; *ICD*, implantable cardioverter defibrillator; *IDC*, idiopathic dilated cardiomyopathy; *IBBB*, incomplete bundle branch block; *IRBBB* or *ILBBB*, right or left IBBB, respectively; *IVCD*, interventricular conduction delay; *LBBB*, left bundle branch block; *MVP*, mitral valve prolapse; *NSR*,

normal sinus rhythm; *NSSTT*, nonspecific ST-T changes on ECG; *NSVT*, non-sustained ventricular tachycardia; *PACs*, paroxysmal atrial contractions; *PM*, pacemaker; *PSVT*, paroxysmal supraventricular tachycardia; *PVCs*, premature ventricular contractions; *RBBB*, right bundle branch block; *SCD*, sudden cardiac death; *tachy*, sinus tachycardia; *SSS*, sick sinus syndrome; *VSD*, ventricular septal defect; *VT*, ventricular tachycardia; *Z*, Z-score of left ventricular end-diastolic dimension.

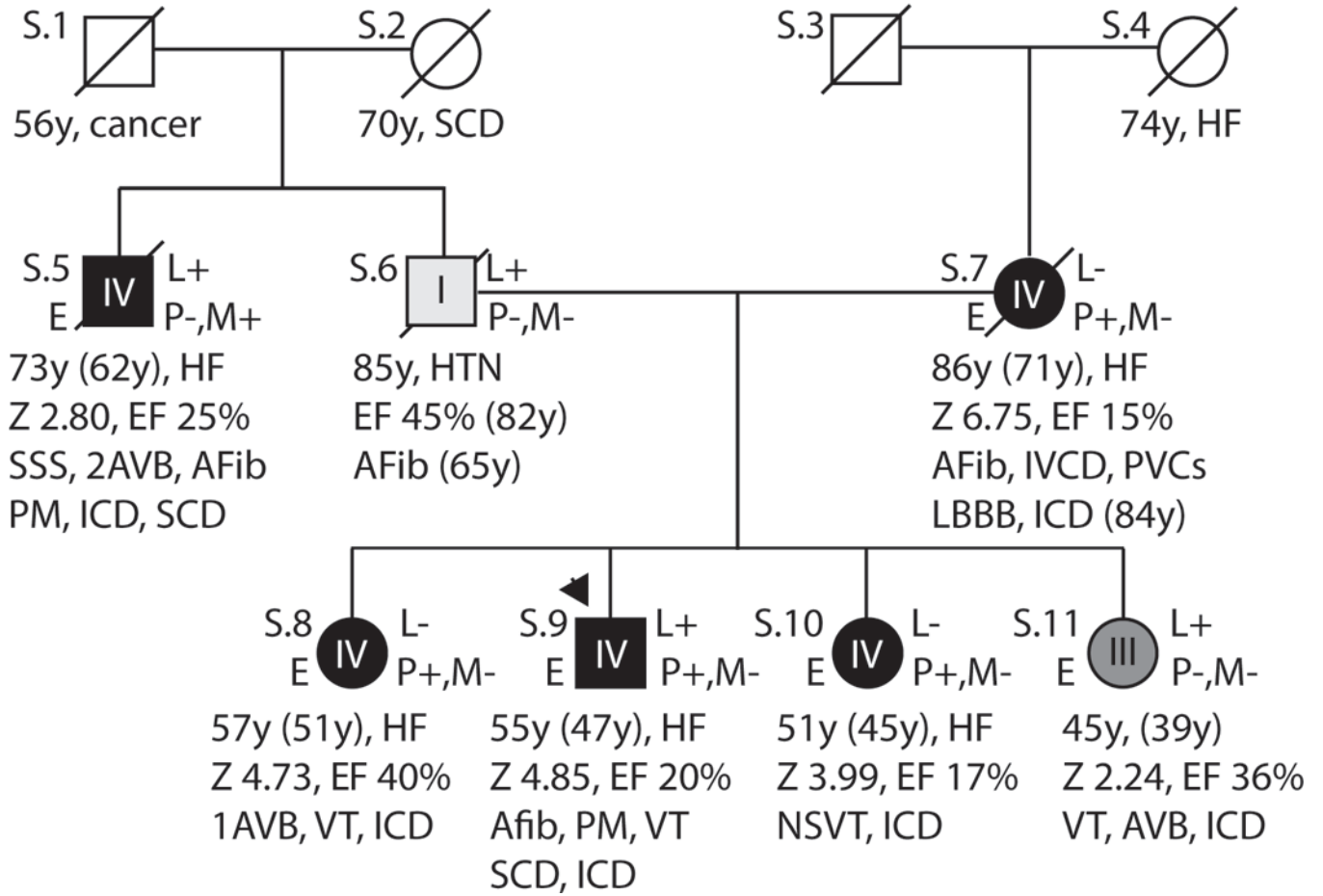
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Pedigree S - LMNA (L), PLN (P), MYPN (M)



**Figure 5: Pedigrees with non-segregating LMNA variants, Pedigree S.**

As per convention, the proband for each family is indicated by an arrow. Males are shown as squares and females as circles. Deceased individuals are denoted by a diagonal slash. Individuals for whom exome sequencing was completed are marked on the left side with an E. Genetic testing results are indicated on the right side by either a + (variant present) or a - (variant absent). Gene abbreviations are provided in the title of each pedigree. Obligate carriers are marked in parentheses (+). The upper right symbol reports genetic testing results for *LMNA*, while the bottom right symbols report testing results for additional identified loci (Table 2). Clinical data is structured as previously described<sup>19</sup>. First line: current age if the individual is still living or age of death if the individual is deceased, age of onset (in parentheses), major diagnosis or cause of death. Second line: age of transplantation, if applicable. Third line: left ventricular size defined by magnitude of standard deviation (Z-score, z) and function defined by left ventricular ejection fraction (EF, %). Fourth line: arrhythmias or other conduction system disease. Abbreviations: *AFib*, atrial fibrillation; *Asym*, asymptomatic; *AVB* atrioventricular block; *1AVB* or *2AVB*, first-degree or second-degree AVB, respectively; *A/W*, alive and well; *BiFB*, bifascicular block; *Br*, bradycardia; *CAD*, coronary artery disease; *CHD*, congenital heart disease; *CM*, cardiomyopathy,

unknown type; *CVD*, cardiovascular disease; *DCM*, dilated cardiomyopathy; *EF*, ejection fraction; *HF*, heart failure; *HTN*, hypertension; *HtTr*, heart transplant; *ICD*, implantable cardioverter defibrillator; *IDC*, idiopathic dilated cardiomyopathy; *IBBB*, incomplete bundle branch block; *IRBBB* or *ILBBB*, right or left IBBB, respectively; *IVCD*, interventricular conduction delay; *LBBB*, left bundle branch block; *MVP*, mitral valve prolapse; *NSR*, normal sinus rhythm; *NSSTT*, nonspecific ST-T changes on ECG; *NSVT*, non-sustained ventricular tachycardia; *PACs*, paroxysmal atrial contractions; *PM*, pacemaker; *PSVT*, paroxysmal supraventricular tachycardia; *PVCs*, premature ventricular contractions; *RBBB*, right bundle branch block; *SCD*, sudden cardiac death; *tachy*, sinus tachycardia; *SSS*, sick sinus syndrome; *VSD*, ventricular septal defect; *VT*, ventricular tachycardia; *Z*, Z-score of left ventricular end-diastolic dimension.



**Table 1.**

## Categories for Clinical Grading of DCM Presence and Severity

Grade	Definition	Comment
Grade 0	No cardiovascular disease, or no cardiovascular abnormalities related to DCM	
Grade 1	Left ventricular enlargement (LVE) without systolic dysfunction, or systolic dysfunction [Left ventricular ejection fraction (LVEF) < 50%] without LVE.	Grade 1 is early evidence of DCM but not meeting formal criteria for a DCM diagnosis.
Grade 2	Asymptomatic DCM.	DCM is defined as systolic dysfunction (LVEF < 50%) and LVE with other usual clinically detectable causes of DCM excluded. In most cases asymptomatic DCM is identified serendipitously or via cascade clinical screening of at-risk family members.
Grade 3	Symptomatic DCM with or without medical therapy.	Most commonly DCM presents with heart failure (80%), less commonly with arrhythmia (15–20%).
Grade 4	DCM requiring advanced interventions (heart transplant, ventricular assist device (VAD), implantable cardioverter defibrillator (ICD) for life threatening symptomatic disease), or DCM-related death.	Advanced interventions are used for late-phase, life-threatening DCM.

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Table 2.

## Summary of Genetic Findings

Pedigree	Gene	RefSeq	UCSC Genomic Position (GRCh37/hg19)	Nucleotide Change *	Amino Acid Change	Max ExAC MAF <sup>†</sup>	Max gnomAD MAF <sup>‡</sup>	dbSNP	Bilineal Inheritance
L	<i>LMNA</i>	NM_170707.3	chr1:156105707	c.952G>A	p.(Ala318Thr)	0	0.00003984 (NFE)	rs267607574	
	<i>BAG3</i>	NM_004281.3	chr10:121429393	c.211C>T	p.(Arg71Trp)	0.0001348 (EAS)	0.0001590 (EAS)	rs387906874	Possible versus de novo
	<i>RBM20</i>	NM_001134363.2	chr10:112541380	c.1013T>C	p.(Met338Thr)	0	0.00003519 (NFE)	rs876657970	
	<i>TTN</i>	NM_001267550.2	chr2:179437290	c.73568del	p.(Pro24523Hisfs*4)	0	0	n/a	
N	<i>DSP</i>	NM_004415.3	chr6:007585972	c.8477G>A	p.(Arg2826His)	0.000100624 (AFR)	0	rs548754771	
	<i>LMNA</i>	NM_170707.3	chr1:156106010	c.1163G>A	p.(Arg388His)	0.00001526 (NFE)	0.00006498 (SAS)	rs267607576	Possible
	<i>RBM20</i>	NM_001134363.2	chr10:112572302	c.2205G>A	p.(Arg716Gln)	0.0005057 (SAS)	0.0003506 (SAS)	rs375798246	
	<i>LMNA</i>	NM_170707.3	chr1:156106042	c.1195C>T	p.(Arg399Cys)	0.00001519 (NFE)	0.00005808 (EAS)	rs58672172	Yes
P	<i>TTN</i>	NM_001267550.2	chr2:179438190	c.72669del	p.(Asp2424Hlefs*8)	0	0	rs727504531	
	<i>LMNA</i>	NM_170707.3	chr1:156106142-156106143	c.1304_1307dup	p.(Ser437Hisfs*2)	0	0.00003251 (SAS)	rs267607577	Presumptive
	<i>NEXN</i>	NM_144573.3	chr1:78401709	c.1453G>A	p.(Glu485Lys)	0.00008684 (AMR)	0.0001108 (NFE)	rs368812830	
S	<i>LMNA</i>	NM_170707.3	chr1:156108540	c.1960C>T	p.(Arg654*)	0.000026 (NFE)	0.00003698 (NFE)	rs267607544	
	<i>PLN</i>	NM_002667.3	chr6:118880157	c.73C>T	p.(Arg25Cys)	0.00001501 (NFE)	0.00008935 (AMR)	rs761056344	Yes
	<i>MYTN</i>	NM_001256267.1	chr10:69881503	c.308A>T	p.(Asp103Val)	0.000172801 (AMR)	0.0001489 (AMR)	rs758026056	

\* By our application of the ACMG approach, all variants were classified as variants of uncertain significance<sup>2,3</sup>

<sup>†</sup>The maximum non-Finnish minor allele frequency (MAF) and associated population for each variant is reported. A MAF of 0 implies absence of the variant from ExAC/gnomAD and sufficient coverage of the genomic position. Reported gnomAD results include both exomes and genomes. Abbreviations: AFR, African; AMR, Latino; EAS, East Asian; NFE, non-Finnish European; SAS, South Asian

**Table 3.**

Descriptive Statistics for the 88 Individuals in the pedigrees in Figures 1–5.

Variable	Values Available	N (%) or Mean $\pm$ Standard Deviation (Minimum, Maximum)
Alive	84	51 (61%)
Female	88	43 (49%)
<i>LMNA</i> variant positive	50	25 (50%)
# of relevant variants in other DCM genes	44	
0		14 (32%)
1		28 (64%)
4		2 (5%)
Phenotypic grade	48	2.1 $\pm$ 1.6 (0, 4)*
0		13 (27%)
1		9 (19%)
2		3 (6%)
3		8 (17%)
4		15 (31%)
Age at echocardiogram (years)	45	41.0 $\pm$ 17.5 (15, 82)
LVEF (%)	44	41.7 $\pm$ 18.8 (8, 70)
LVEDD (z-score)	40	2.64 $\pm$ 2.08 (–3.09, 6.75)

Includes parents of siblings O.3 and O.5 as well as siblings P.1, P.3, and P.6. These 4 parents did not contribute variant or phenotype data and so were not displayed on pedigrees to save space. Some percentages may not sum to exactly 100 because of rounding. DCM indicates dilated cardiomyopathy; LVEDD, left ventricular end-diastolic dimension; and LVEF, left ventricular ejection fraction. \*Phenotypic grade is defined as a categorical variable but used as a quantitative trait in analysis.

**Table 4.**

## Multivariate Linear Mixed Model Results

	LVEF (%)	LVEDD (z-score)	Phenotypic grade
	<b>Mean model parameters</b> <i>Estimate ± Standard Error</i> (95% Confidence Interval)*		
Intercept	49.19 ± 6.18 (37.07, 61.30)	2.24 ± 0.67 (0.93, 3.55)	1.55 ± 0.46 (0.63, 2.46)
Female	3.47 ± 5.35 (-7.01, 13.95)	-0.24 ± 0.64 (-1.50, 1.02)	-0.09 ± 0.46 (-0.99, 0.82)
Age at echocardiogram (standardized)	-6.26 ± 2.92 (-11.98, -0.55)	0.50 ± 0.34 (-0.17, 1.17)	0.74 ± 0.24 (0.28, 1.21)
LMNA variant positive	3.38 ± 5.57 (-7.54, 14.30)	-1.19 ± 0.65 (-2.46, 0.08)	-0.57 ± 0.47 (-1.49, 0.35)
At least one relevant non-LMNA variant in other DCM genes	-12.62 ± 5.27 (-22.96, -2.29)	1.44 ± 0.63 (0.19, 2.68)	0.82 ± 0.45 (-0.07, 1.71)
	<b>Additive genetic covariance components<sup>†</sup></b> <i>Estimate ± Standard Error</i>		
LVEF (%)	87.81 ± 578.79	-5.94 ± 13.43	-4.85 ± 21.78
LVEDD (z-score)	-5.94 ± 13.43	0.40 ± 1.64	0.33 ± 1.02
Phenotypic grade	-4.85 ± 21.78	0.33 ± 1.02	0.27 ± 1.01
	<b>Environmental covariance components<sup>†</sup></b> <i>Estimate ± Standard Error</i>		
LVEF (%)	117.38 ± 105.05	-10.26 ± 10.33	-9.62 ± 7.22
LVEDD (z-score)	-10.26 ± 10.33	2.28 ± 1.16	1.28 ± 0.76
Phenotypic grade	-9.62 ± 7.22	1.28 ± 0.76	1.20 ± 0.56

\* Wald 95% confidence intervals were calculated from the reported estimate and standard error using the standard normal distribution.

<sup>†</sup> Standard errors for covariance components were calculated using formula (10) of Bauman et al.<sup>25</sup>, which is reported as STD\_ERR\_2 by the Mendel software.