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Clinical and Genetic Investigations of 109 Index-patients With Dilated Cardiomyopathy and 445 of Their Relatives

Short Title: Clinical and Genetic Investigations in DCM

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ABSTRACT

BACKGROUND

It was the aim to investigate the frequency and genetic basis of dilated cardiomyopathy (DCM) among relatives of index-patients with unexplained heart failure (HF) at a tertiary referral center.

METHODS AND RESULTS

Clinical investigations were performed in 109 DCM index-patients and 445 of their relatives. All index-patients underwent genetic investigations of 76 disease associated DCM-genes.

A family history of DCM occurred in 11% (n=12) while clinical investigations identified familial DCM in a total of 32% (n=35). One fifth of all relatives (n=95) had DCM of whom 60% (n=57) had symptoms of HF at diagnosis while 40% (n=38) were asymptomatic. Symptomatic relatives had a shorter event-free survival than asymptomatic DCM-relatives ($p<0.001$).

Genetic investigations identified 43 pathogenic (P) (n=27) or likely pathogenic (LP) (n=16) variants according to ACMG-criteria. 44% (n=48/109) of index-patients carried a P/LP-variant of whom 36% (n=27/74) had sporadic DCM while 60% (21/35) were familial cases. Thirteen of the P/LP -variants were also present in ≥7 affected individuals and thereby considered to be of sufficient high confidence for use in predictive genetic testing.

CONCLUSION

A family history of DCM identified only 34% (n=12/35) of hereditary DCM, while systematic clinical screening identified the remaining 66% (n=23) of DCM families. This emphasized the importance of clinical investigations to identify familial DCM. The high number of P/LP variants identified in familial DCM provide a firm basis for offering genetic investigations in affected families. This should also be considered in sporadic cases since adequate family evaluation may not

always be possible and the results of the genetic investigations may carry prognostic information with an impact on individual management.

KEYWORDS

Dilated cardiomyopathy, Genetic investigations, Family screening, Genetic counseling

NON-STANDARD ABBREVIATIONS AND ACRONYMNS

ACMG = the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

BSA = body surface area

CA = cardiac arrest

DCM = dilated cardiomyopathy

gnomAD = Genome Aggregation Database

HF = heart failure

HTx = heart transplantation

IQR = interquartile range

IVA = Ingenuity Variant Analysis

LP = Likely pathogenic

LV = left ventricle

LVEDd = left ventricular end-diastolic diameter

LVEF = left ventricular ejection fraction

NGS = next generation sequencing

P = Pathogenic

SCD = sudden cardiac death

VA = ventricular arrhythmias

VF = ventricular fibrillation

VT = ventricular tachycardia

VUS = variant of unknown significance

INTRODUCTION

Dilated cardiomyopathy (DCM) is a condition characterized by unexplained dilation and impaired systolic function of the left ventricle (LV).[1, 2] The most common presentation is with symptoms of heart failure (HF), although stroke, severe arrhythmias and sudden cardiac death (SCD) may be the first manifestations of the disease. Previous studies have suggested that DCM is familial in 20-40% of cases and that genetic investigations may identify a causative mutation in 35-45% of index-patients with hereditary DCM.[3-5]

In this study we present the results of comprehensive clinical and genetic investigations of both DCM index-patients and their relatives at risk of having inherited the condition. The aims were to (1) determine the frequency of familial DCM in a cohort of index-patients at a tertiary referral center, (2) investigate if affected asymptomatic relatives identified by clinical family investigations had a better prognosis than relatives presenting themselves with symptoms and (3) to identify DNA sequence variants that could be used for predictive genetic testing in affected families by performing co-segregation analysis.

METHODS

Study Design and Patient Cohort

The data that support the findings of this study are available from the corresponding author upon request. This investigation was conducted in accordance with the declaration of Helsinki and approved by the local ethics committee (S-20140073) as well as the Danish data protection agency (14/17347). Informed consent was obtained from all participants of the study.

The study cohort consisted of consecutive and unrelated index-patients with a diagnosis of DCM who were followed at a University hospital with a cardiac transplant program (HTx). They were either diagnosed within the local catchment area of the University Hospital or referred from regional hospitals for follow-up and included in the study from January 2011 until December 2012. Clinical data of the index-patients were collected retrospectively from their initial diagnosis of DCM until inclusion in the study and from then, prospectively until their most recent follow-up, their time of death or HTx.

A pedigree with at least three generations was drawn upon evaluation of each index-patient. All relatives at risk of having inherited the condition were offered systematically serial clinical investigations from the age of 13 consisting of a physical examination, echocardiography, and ECG-recording.[6] Relatives with normal clinical investigations were offered prospective follow-up every third year. Patients diagnosed with DCM were followed on a yearly basis according to standard guidelines and at the physician's discretion.[2] Clinical evaluation of relatives was performed at the study center. Information about family members who were diagnosed or had died before the current investigations was obtained by reviewing available hospital notes and autopsy reports. Familial DCM was defined as the appearance of more than one affected individual (deceased or alive) with a confirmed diagnosis of the condition.

The following information was obtained of each individual included in the investigation: (1) symptoms of cardiac disease, (2) results of ECG and Holter-recordings, (3) results of echocardiography, (4) implantation of pacemaker or defibrillator, (5) disease complications including ventricular arrhythmias (VAs), HTx and (6) all-cause mortality.

Ventricular Arrhythmia (VA)

VA was defined as documented episodes of (a) sustained ventricular tachycardia (VT) lasting >30 seconds requiring cardioversion, (b) ventricular fibrillation (VF) or (c) SCD. Incidents of VF and VT requiring cardioversion were recorded by ICD or ECG.

Sudden Cardiac Death

SCD was defined as a natural, sudden and unexpected death. In un-witnessed cases SCD was defined as the cause of death in a person last seen alive and functioning normally 24 hours before dying and in witnessed cases as an acute change in cardiovascular status within one hour of the time to death.[7]

DCM Diagnostic Criteria

DCM was diagnosed when echocardiography identified unexplained left ventricle dilation and impaired contractile performance with a left ventricular end-diastolic diameter (LVEDd) >112% predicted for age and body surface area (BSA) and a left ventricular ejection fraction (LVEF) <45%.[2, 8, 9]

Coronary artery disease was excluded in index-patients ≥35 years of age by coronary angiography or standard coronary computer tomography (CT) scan.[2] In addition, index-patients with HF and reduced LVEF who were pregnant, had hypertension, a history of alcohol abuse, heart valve disease, congenital heart disease, autoimmune, endocrine, metabolic or neuromuscular disease were excluded from the study.

Relatives who died from SCD <50 years of age and carried a disease-associated pathogenic (P)/ likely pathogenic (LP) variant or had an autopsy performed consistent with a diagnosis of unexplained HF were also considered to have DCM.[2]

Echocardiography

All individuals underwent echocardiography which included standard two-dimensional measurements of LVEDd and LVEF by Simpson's bi-plane method. Dimensions were corrected for age and BSA according to the formula of Henry [(LVEDd) (percent predicted) = measured LVEDd/predicted LVEDd X 100; predicted LVEDd = $[45.3 \times BSA^{0.3}] - [0.03 \times age] - 7.2$.[9]

Genetic Investigations

All index-patients underwent genetic investigation by Next Generation Sequencing (NGS) in 76 known or candidate DCM genes by use of Illumina Hiseq NGS technology as reported previously (Supplemental Table 1).[10] The quality of the sequencing data has also been reported previously.[10] Raw data from the NGS was processed and annotated as described in the supplemental material. Once a likely P or LP sequence variant considered useful for genetic counseling was identified in index-patients their relatives were offered predictive genetic testing by use of Sanger Sequencing.

Filtering and Classification of DNA-Sequence-Variants

Sequence variants were filtered and classified according to the consensus recommendations from the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG) as P, LP, variant of unknown significance (VUS), benign or likely benign.[11] The initial raw filtering and classification was obtained by use of the software Ingenuity Variant Analysis (IVA, Qiagen®) and InterVSar (Wang Genomics Lab®).[12, 13] Subsequently, all P/LP variants as well as VUS were reassessed individually and reclassified according to ACMG criteria, following co-segregation analysis, literature- and database- review.[14-17] Evidence of segregation

was considered as strong when appearing in ≥7 affected individuals, moderate when appearing in ≥5 and supporting when appearing in ≥3.[18]

Variants classified as P/LP according to ACMG criteria were considered to be of sufficient high confidence for use in predictive genetic testing when they in addition:

(a) Appeared in ≥ 7 individuals fulfilling DCM diagnostic criteria based on the findings in the current study and previous reports.[14-18]

(b) Occurred with an allele frequency <1:25.000 in the Genome Aggregation Database (gnomAD) using the global allele frequency. [18, 19]

Statistics

Continuous variables were reported as mean ± standard deviation for normally distributed data or otherwise as median and 25-75% interquartile range (IQR). Categorical variables were reported with numbers and percentages. Comparisons of groups were made by use of the student's t-test or Wilcoxon rank-sum test when appropriate for continuous variables and the $\chi 2$- or Fishers exact-test were used when appropriate for categorical variables. The follow-up period in the survival analyses lasted from date of birth to the date of HTx, death from any cause, episode of sustained ventricular tachycardia requiring cardioversion or ventricular fibrillation (composite endpoint), or the date of the most recent follow-up (censoring). The event-free survival probability was estimated using the Kaplan-Meier method, were compared using the log-rank test. A p-value of <0.05 was considered to indicate a significant difference. Statistical analyses were performed by the use of software from StataCorp (version Stata/IC 15.0).

RESULTS

Clinical investigations

Index-patients

One hundred-nine index-patients, (76 males; 33 females), were diagnosed with DCM due to symptoms of HF at an average age of 38.8 ± 15.7 years and followed for a median period of 60 months (IQR: 25 – 106 months) (Table 1). Fifty index-patients had received a cardiac transplant at the time of inclusion in the study while eight had survived a cardiac arrest (CA) as their initial manifestation of DCM.

Clinical Characteristics of Index-patients With Familial or Sporadic DCM

Twelve (11%) index-patients had a positive family history of DCM upon drawing the pedigree while systematic clinical investigations identified an additional 23 DCM families which resulted in a total of 35 index-patients (32%) with familial DCM. The remaining 74 index-patients (68%) had no affected relatives and therefore assumed to be sporadic cases (Figure 1A). In 12 of the patients assumed to have sporadic DCM no relatives were available for clinical investigation either because they declined the offer (n=4) or had died (n=8). In total, the family history with information about the presence of additional DCM relatives identified 34% of the familial cases while the vast majority of 66% were identified following clinical family screening.

The clinical characteristics of index-patients with familial and sporadic DCM were compared in order to identify potential predictors for hereditary DCM. However, there were no apparent differences regarding age at diagnosis, clinical characteristics and outcome between the two groups of index-patients (Figure 2A, Supplemental Figure 1A and Table 1). Although index-patients with sporadic DCM had a significant lower LVEF than individuals with familial DCM there was no difference when comparing LVEDd and symptoms of cardiac disease between the two groups (Table 1).

Relatives

Approximately 475 relatives were offered clinical investigations via the index-patients of whom 445 (94%) accepted the offer (Figure 1A). Two hundred and twenty-eight (51%) were females. The number of affected male relatives (26%; n=56/217) was significantly higher than affected female relatives (17%; n=39/228) (p=0.025), while the number of affected female relatives with symptoms at diagnosis (62%; n=24/39) was the same as in affected male relatives (60%; n=33/56) (p=0.80). Three hundred and fifty relatives (79%) had a normal cardiac investigation at an average age of 41.5 ± 18.8 years (Figure 1A). Ninety-five relatives (21%) were diagnosed with DCM at an average age of 38.7 ± 16.6 years (Table 2 and Supplemental Figure 2). Forty-three (45%) of these individuals had previously been referred for cardiac investigations due to cardiac symptoms, while 12 (13%) had died suddenly and two (2%) survived a CA as their initial symptom of disease (Table 2, Figure 1A). Nine of these deceased individuals had an autopsy performed, which was consistent with a diagnosis of DCM. However, there were no medical data available in the remaining three sudden deaths, which all turned out to have relatives with DCM and shown to be obligated carriers of recognized disease-associated P/LP variants following subsequent genetic investigations.
The remaining 38 affected relatives (40%) were diagnosed with DCM following family screening (n=31) or developed DCM during follow-up (n=7). None of these individuals had any symptoms of cardiac disease at diagnosis (Table 2, Figure 1A). Some of the symptomatic relatives were unknown to the index-patients and occurred with the same frequency and independent of whether the family history of DCM was positive (33%; n=4/12) or negative (35%; n=8/23).

Clinical Characteristics of Relatives With DCM

Relatives who were diagnosed with DCM due to symptoms of HF were significantly older than relatives identified with DCM following family screening (41.8 ± 16.3 vs. 33.9 ± 16.2 years, p=0.023). In addition, they had a significantly poorer LVEF (26 ± 11 vs. 38 ± 8 %, p <0.001) and larger LVEDd (66 ± 9 vs. 60 ± 6 mm, p=0.002) (Table 2). In both groups, the mean LVEDd

remained unchanged during follow-up. However, the mean LVEF improved significantly from diagnosis to the most recent echocardiography among relatives with DCM who were asymptomatic at diagnosis (38 ± 8 vs. 43 ± 11%, p=0.010).

Affected relatives who were symptomatic at diagnosis had a higher frequency of adverse disease complications including more incidents of VAs and death from any cause (Table 2). In addition, they also had a significantly shorter event-free survival compared to affected relatives who were asymptomatic at diagnosis, when including VAs (23% (n=21/57) vs. 3% (n=1/38)), HTx (18% (n=8/45) vs. 5% (n=5/38)) and death among non-SCD/non-HTx patients (38% (n=14/34) vs. n=0)) in the analyses (p <0.001) (Table 2, Figure 2B and Supplemental Figure 1B). This difference remained the same even when symptomatic individuals who died suddenly or survived a CA as their first symptom of disease were omitted from the analyses (Supplemental Figure 3). Apparently, the results of the survival analyses were not caused by differences in sex, medical treatment or pacemaker therapy received by the two groups (Table 2).

Age Distribution at Diagnosis of Individuals With Familial DCM

As mentioned previously 35 DCM families were identified with 95 affected relatives of whom 90 (95%) were diagnosed within the second to sixth decade of life (Supplemental Figure 2). Three (3%) had onset of disease in their first decade of life while only two (2%) were diagnosed in their seventies.

Genetic Investigations

Mutational analyses of 76 recognized or likely candidate genes for DCM within the 109 index-patients identified 641 VUS (Supplemental Table 2) and a total of 43 different P (n=27) or LP variants (n=16) according to the ACMG-classification (Table 3 and Supplemental Table 3). A total of 48 index-patients carried a P/LP variant of whom 27 turned out to have sporadic DCM while 21 were familial cases (Figure 1 B). All of the P/LP variants appeared with a frequency <1:25.000 in

the GnomAD database (Table 3[4, 20-34], Supplemental Table 3, 4 and 5). Forty-four percent (n=48/109) of index-patients carried at least one variant classified as P/LP. The frequency of P/LP variants was significantly higher among index-patients with familial DCM compared to sporadic cases (60% (n=21/35) vs. 36% (n=27/74)) (p=0.021) (Figure 1B). P/LP variants within the gene for *TTN* were most prevalent with a frequency of 15% among index-patients (n=16/109). Two index-patient carried multiple P/LP variants, that did not allow for predictive genetic testing of sufficient high confidence (Supplemental Table 3). The first of these two carried variants in genes for *ACTC1* and *RYR2* (Supplemental Table 3). She was diagnosed at the age of 26 following successful resuscitation from a CA. At the age of 47 her condition stabilized with an LVEF of 35% and she has received no therapies from the ICD implanted following her CA. Two of her relatives had DCM and carried the same *ACTC1* variant, while no one else carried the *RYR2* variant.

The second index-patient carried two different P-variants in *TTN* (genomic position: 179474220 and 179476569; Supplemental Table 3). He was diagnosed with DCM at the age of 25 with a LVEF of 15% and underwent HTx due to end-stage heart failure at the age of 31. The variants were carried in cis since his father and twin-sister carried both variants. However, they had no signs or symptoms of disease at the age of 64 and 37, respectively.

Thirty of the 43 (70%) P/LP variants were identified in less than seven individuals with DCM in this or previous studies. Therefore, they were not considered to be of sufficient high confidence for use in predictive genetic testing (Table 3 and Supplemental Table 3). The remaining 13 P/LP variants were of sufficient high confidence to be used for predictive genetic testing and were identified in a total of 18 index-patients (17%; n=18/109) (Table 3). Two of these variants were novel and identified in the genes for *TPM1* and *TTN* (Supplemental Figure 4). The high-confidence P-variants were most frequently identified in *RBM20* (6%; n=7/109), *LMNA* (5%; n=5), sarcomeric protein genes (4%; n=4), and *TTN* in (2%; n=2).

A total of 132 carriers of high-confidence P-variants were identified following the genetic investigations including 18 index-patients and 114 of their relatives at risk of having inherited DCM. Ninety were diagnosed with DCM at their initial investigation (n=86) or developed the condition (n=4) during a median follow-up of 64 months (IQR: 14-122). The average age at diagnosis was 36.5 ± 15.5 years while 42 unaffected carriers had an age of 30.4 ± 15.6 years at their most recent follow-up.

The penetrance among carriers including index-patients of high-confidence P-variants was 89% (n=8/9) in *TTN*, 79% (n=22/27) in sarcomeric genes, 68% (n=38/56) in *RBM20*, followed by 55% (n=22/40) in *LMNA*. The mean age in years at most recent clinical evaluation of healthy carriers was; 65.5 (*TTN;* n=1), 21.7±12.1 (sarcomere genes; n=5), 34.0 ± 15.8 (*RBM20*; n=18) and 27.4 ± 13.4 (*LMNA*; n=18) (Table 3).

High-Confidence P-Variants Used for Predictive Genetic Testing in Familial DCM

The majority of high-confidence P-variants suitable for predictive genetic testing was identified in index-patients with familial DCM (46%; n=16/35) (Figure 1B). There was no difference in the relative number of high-confidence P-variants suitable for predictive genetic testing identified among those with a positive (42%; n=5/12) or negative family history (48%; n=11/23) (p = 0,74). Most of the affected individuals carried high-confidence P-variants in either *RBM20* (n=38) or *LMNA* (n=22). Both of these groups had an apparently high frequency of VAs (Table 4). Although carriers of high-confidence P-variants within *RBM20* were significantly younger at diagnosis than carriers of high-confidence P-variants within *LMNA* (35.4 ± 13.6 vs. 45.9 ± 12.3 years, $p<0.01$) there were no additional differences between the clinical characteristics of the two groups (Table 4). The number of affected individuals in families with high-confidence P-variants in *TTN* (n=8), *MYH7* (n=7), *TNNT2* (n=7) and *TPM1* (n=8) was too small for detailed comparisons of genotype-phenotype relations.

Genetic Investigations of Individuals With Sporadic DCM

Twenty-seven index-patients with sporadic DCM carried at least one P/LP variant according to ACMG criteria. However, only two (3%; n=2/74) were shown to carry high-confidence P-variants suitable for predictive genetic testing (Figure 1B and Supplemental Figure 4). In Family 5, individual II-2 was diagnosed with DCM at the age of nine years and carried a high-confidence P-variant within *TNNT2* (p.Arg173Gln/c.518G>A), which had previously been reported in ten DCM patients.[32-34] Due to the early and severe onset of disease and the absence of additional affected individuals in the family the variant may have arisen de novo although definitive proof would have needed further genetic investigation of the parents.

In Family 4, individual II-1 was diagnosed with DCM at the age of 51 and shown to carry the same high-confidence P-variant in *TTN* as identified in Family 1 (Supplemental Figure 4). No relatives were available for clinical investigations and therefore it was not possible to establish if the variant had been inherited or arisen de novo. An additional 11 sporadic DCM index-patients did not have relatives available for the study. The genetic investigations of these patients did not identify any P/LP variants.

DISCUSSION

Limited Sensitivity of Family History and Clinical Characteristics to Identify Hereditary DCM

The study cohort consisted of 109 DCM index-patients and 445 of their relatives who all underwent clinical investigations to establish the frequency of familial DCM which was shown to be 32% in consistency with previous findings.[8]

The accuracy of the family history to identify hereditary DCM was investigated following construction of a three-generation pedigree of each family based on information from the index-patient, which included the presumed health status of relatives. Surprisingly, despite systematic, detailed pedigree construction, family history had limited sensitivity in identifying familial disease. Indeed, in 66% of index patients with no family history of DCM, familial disease was identified only after clinical investigations of relatives. These findings highlight the limitations of gathering family history as many patients may not be aware of medical details in their family, or even in communication with informative relatives. Although the family history had a low predictive value in identifying hereditary DCM it is still our practice to draw a pedigree based on information from the index-patient at first. Relevant hospital notes and death certificates are then obtained of relatives from the pedigree and evaluated. Secondly, clinical investigations of at-risk individuals are offered and performed. Based on the results of these investigations genetic testing are offered if at least one additional relative has a confirmed diagnosis of DCM.

The clinical characteristics of index patients with sporadic and familial DCM were also investigated. The only difference identified was a significantly higher LVEF among index-patients with familial DCM than sporadic DCM. However, there were no differences when comparing LVEDd and NYHA functional class between the two groups. Therefore, this finding was considered to be coincidental and would need to be investigated in a different and larger cohort of patients before the validity can be evaluated.

Since no clinical characteristics distinguished hereditary or sporadic DCM and the ascertainment of the family history was of limited accuracy and scope, clinical investigations of at-risk relatives is required to identify familial DCM. Therefore, it is recommended to perform systematic clinical follow-up of at-risk relatives including physical examination, echocardiography and ECG-recording as outlined in the methods section.[2]

Implications of Family Screening for Relatives

The clinical investigations showed that approximately one fifth of all relatives fulfilled DCM diagnostic criteria of whom 45% had symptoms of HF, 40% were asymptomatic and diagnosed due to family screening, while 15% had died suddenly or survived a CA as the initial symptom of disease.

Symptomatic relatives were older at diagnosis and more severely affected than asymptomatic relatives. This may reflect that family screening identified asymptomatic individuals at an earlier stage of the disease and that individuals became symptomatic at an older age due to the progressive nature of DCM characterized by a continuous loss of LV function over time. This finding was also likely to explain that symptomatic individuals had a significantly shorter event-free survival than asymptomatic individuals due to their poorer LV-function (Figure 2B). However, it was surprising that asymptomatic relatives continued to have very few events during follow-up and that their LV-function appeared to improve significantly. A possible explanation of this finding was that asymptomatic relatives with DCM received HF treatment at an earlier stage of the disease development than symptomatic individuals who had more pronounced LV impairment before initiating therapy as shown previously (Table 2).[35, 36] Whether presentation of DCM without symptoms represent a milder phenotype with a benign prognosis remain unknown and will be difficult to elucidate further since current guidelines recommend treatment even in asymptomatic HF patients.[6]

The implication of family screening was that asymptomatic relatives with DCM were diagnosed

and treated early in the course of the disease, which may have a beneficial effect on their LV function and event-free survival.

Genetic Architecture of DCM

Genetic investigations are generally recommended in DCM in order to identify P/LP variants that may be used for predictive testing of healthy relatives at risk of developing the condition later in life.[2] The implication of genetic testing is that carriers of the P/LP variants are recommended continuous clinical surveillance while non-carriers will be discharged from further follow-up. Within recent years it has become possible to investigate numerous recognized and likely disease-associated genes in the context of hereditary conditions by use of NGS technology.[5] Such genetic investigations identify a considerable number of DNA variants, which increases with the number and size of the particular genes investigated. It is often difficult to establish when specific DNA variants are pathogenic and can be used for predictive testing and counseling of affected families. Correct interpretation and classification of the DNA variants require several lines of evidence including specialized knowledge in bioinformatics.

In the current study the frequency of P/LP variants according to ACMG criteria was 43%, with *TTN* being the most frequent disease gene accounting for 15% of all P/LP variants identified, which was consistent with previous findings.[4, 37] However, we chose to use DNA sequence variants for predictive genetic testing in affected families only, when they fulfilled ACMG criteria for being P/LP as well as being present in at least seven affected individuals. This last criteria was included since the presence of a specific DNA-variant in many individuals affected by the same condition such as DCM increases the likelihood considerably of the variant being disease associated and thereby ensures correct genetic counselling.[5, 11, 18] By use of this algorithm 17% of index-patients in the study were considered to carry high-confidence P-variants suitable for predictive genetic testing.

These variants were most commonly identified in *RBM20* (6%) followed by *LMNA* (5%) and were associated with a similarly severe disease expression consistent with previous reports.[28, 38] However, it is highly likely, that the gene-specific distribution of high-confidence P-variants will change in the near future as a consequence of the rapidly growing number of genotyped DCM families, which will inevitably identify many more DCM-patients carrying identical P-variants.

The yield of genetic investigations in identifying P/LP variants according to ACMG criteria was significantly higher in familial DCM (60%) compared to sporadic cases (36%). This finding was mirrored in the distribution of high-confidence P-variants used for predictive genetic testing which was 46% in familial DCM and 3% in sporadic cases (Figure 1 B). These results were to be expected, since the presence of more than one affected individual in a family would be suggestive of the hereditary form of DCM and thereby increase the likelihood of identifying P/LP variants including high-confidence variants. The high yield of the genetic investigations in familial DCM provided a firm basis for offering these investigations to affected families. Although the yield was less in sporadic DCM, genetic investigations should still be considered since the result may have prognostic implications by identifying variants in disease genes associated with a severe disease expression such as in *LMNA* and *RBM20*.[28, 38] Such findings may have an impact on prognosis and clinical management of affected individuals. Furthermore, adequate family investigations may not always be possible which imply that hereditary DCM may be missed in apparently sporadic cases.

Limitations

Index-patients were included at a tertiary referral hospital with a transplant program which explained the high frequency of HTx among index-patients and may have introduced selection bias towards more severely affected and younger individuals. However, the frequency of familial disease appeared to be the same as in previous reports from non-transplant centers.[8]

Although all relatives at risk of having inherited DCM were invited to participate in the study some individuals with unknown clinical status declined the offer which may have led to an overestimation of the number of sporadic cases. In addition, the number of relatives who were clinically investigated where higher in familial cases (n=265) compared to sporadic cases (n=180) which may have led to an underestimation of the number of familial cases.

CONCLUSION

Clinical investigations of relatives to index-patients with DCM remain an essential part of the diagnostic workup since family history and drawing of three-generation pedigree was of limited sensitivity in identifying affected relatives. The high yield of identifying P/LP variants by the genetic investigations in familial DCM provided a firm basis for offering these investigations to affected families. This should also be considered in sporadic cases since adequate family evaluation may not always be possible and the results of the genetic investigations may carry prognostic information with an impact on individual management.

WHAT IS NEW?

- Obtainment of a family history and construction of a three-generation pedigree was of limited value in identifying familial DCM, which highlight the need of doing clinical investigations to identify affected relatives
- Clinical investigations identified a significant number of asymptomatic relatives with DCM who were younger with a better LV-function and prognosis compared to DCM-relatives with symptoms at diagnosis who were older with a worse LV-function and outcome

WHAT ARE THE CLINICAL IMPLICATIONS?

- Family screening including clinical evaluation is a cornerstone in the diagnostic work-up of DCM and should be offered to all relatives of index-patients with the condition

- Genetic investigations should be performed in confirmed familial DCM
- Genetic investigations should be considered in sporadic DCM since clinical evaluation of relatives may not rule out a hereditary cause of the condition and the identification of specific P/LP variants may have implications for prognosis and clinical management

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Conflicts of Interest

None declared

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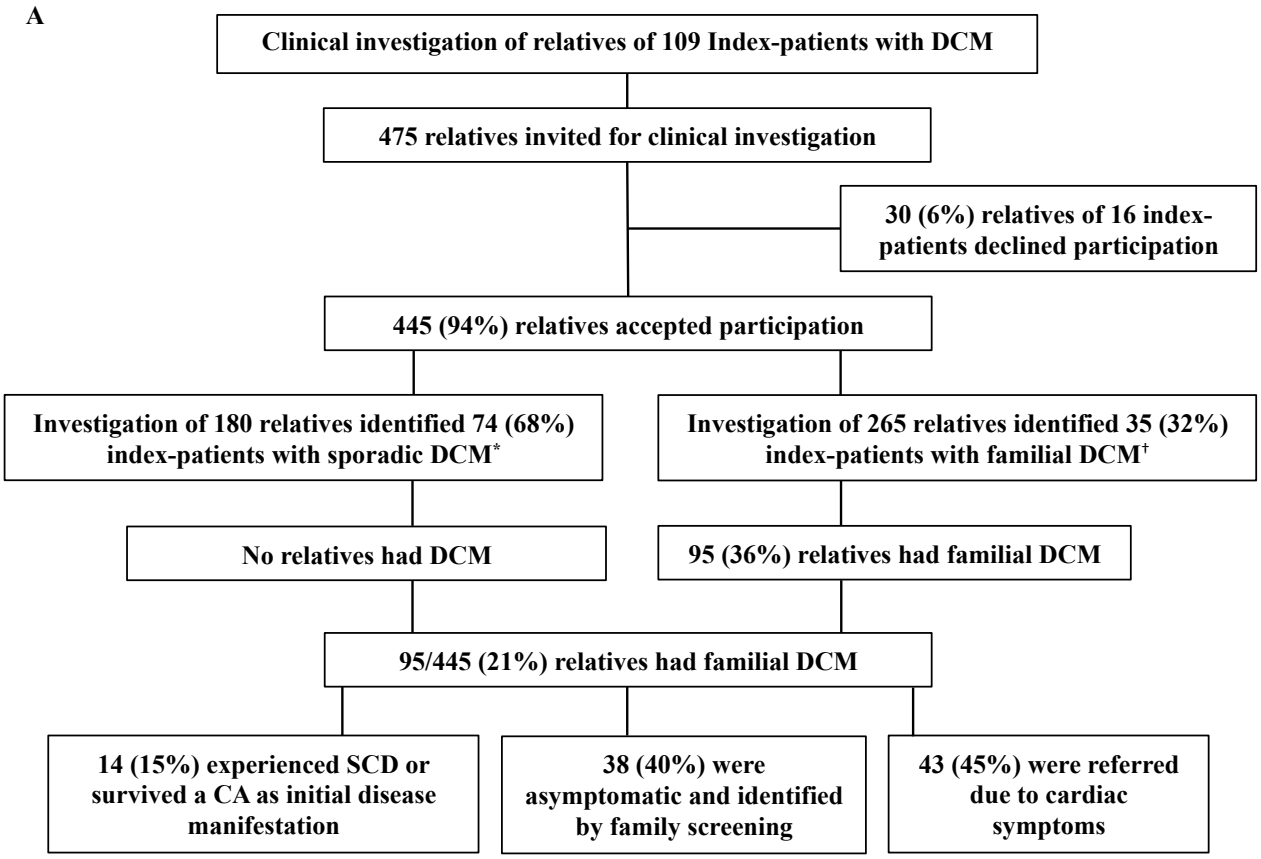
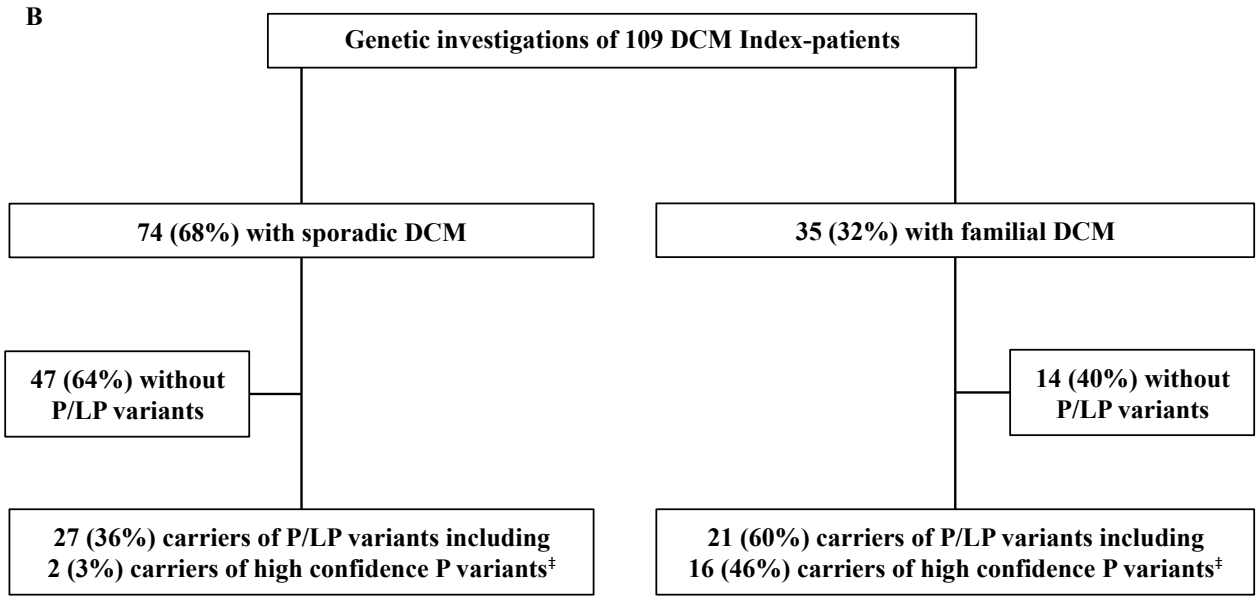
FIGURE LEGENDS

Figure1


A
Clinical investigation of relatives of 109 Index-patients with DCM
475 relatives invited for clinical investigation
30 (6%) relatives of 16 index-patients declined participation
445 (94%) relatives accepted participation
Investigation of 180 relatives identified 74 (68%) index-patients with sporadic DCM*
Investigation of 265 relatives identified 35 (32%) index-patients with familial DCM†
No relatives had DCM
95 (36%) relatives had familial DCM
95/445 (21%) relatives had familial DCM
14 (15%) experienced SCD or survived a CA as initial disease manifestation
38 (40%) were asymptomatic and identified by family screening
43 (45%) were referred due to cardiac symptoms


B
Genetic investigations of 109 DCM Index-patients
74 (68%) with sporadic DCM
35 (32%) with familial DCM
47 (64%) without P/LP variants
14 (40%) without P/LP variants
27 (36%) carriers of P/LP variants including 2 (3%) carriers of high confidence P variants‡
21 (60%) carriers of P/LP variants including 16 (46%) carriers of high confidence P variants‡

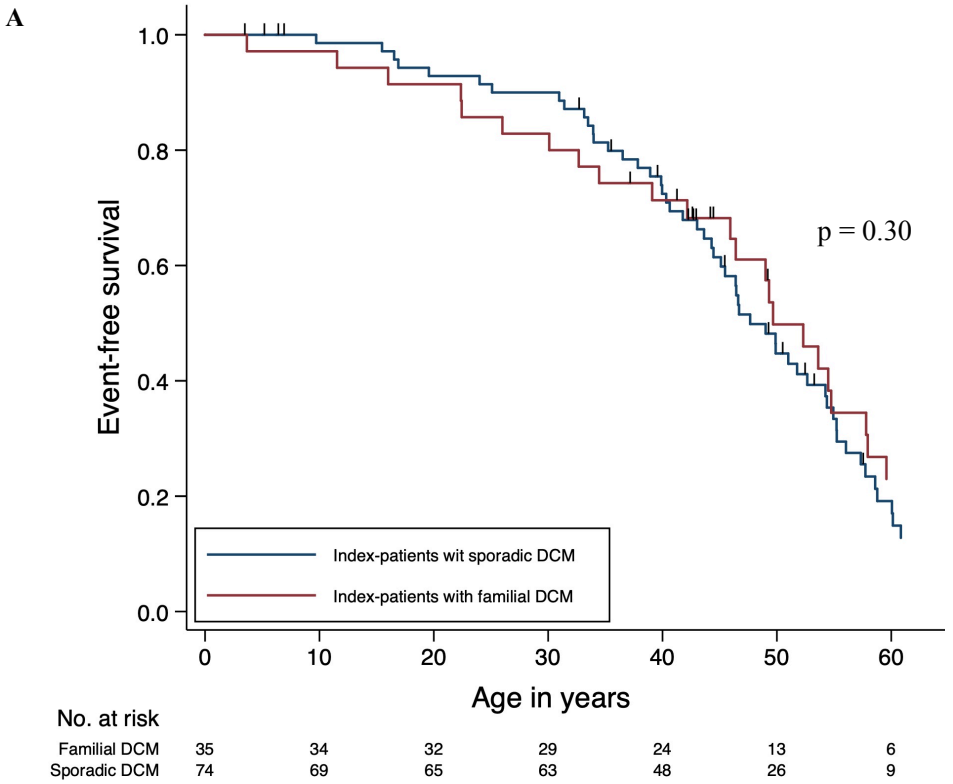
Results of Clinical- (A) and Genetic-investigations (B)

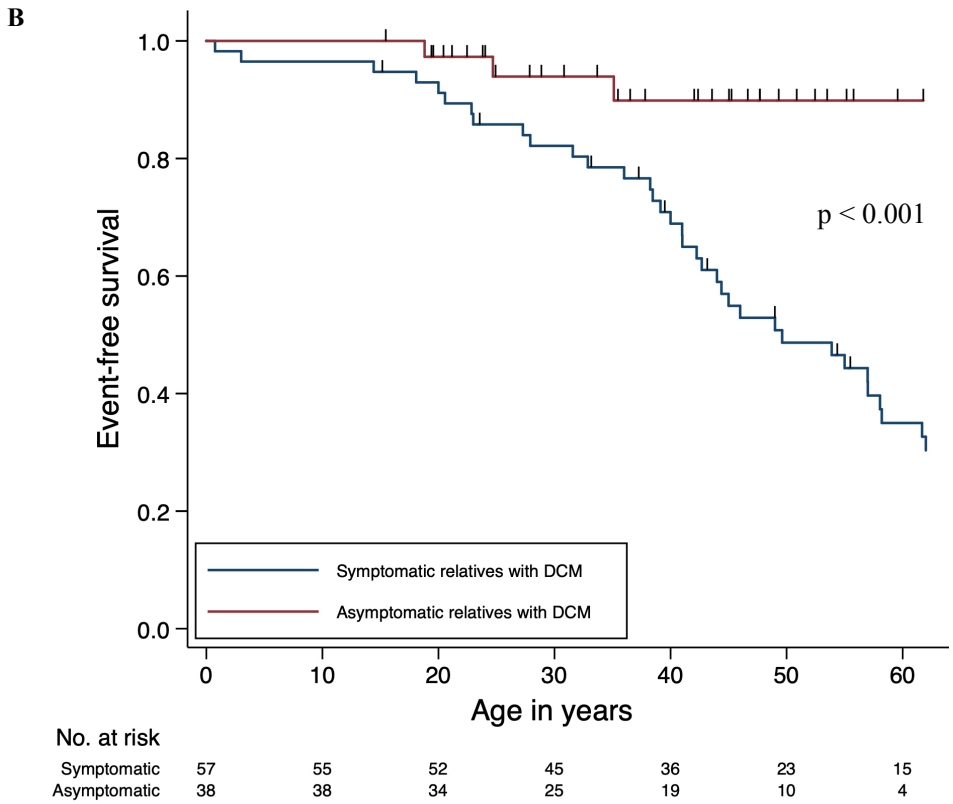
(A) Clinical investigations of 109 index-patients with DCM and 445 of their relatives revealed a familial appearance of the disease in 32% of cases.

(B) Distribution of pathogenic/likely pathogenic variants according to ACMG and high-confidence pathogenic variants among 109 index-patients with either sporadic (n=74) or familial DCM (n=35).

*Pathogenic/Likely pathogenic sequence variants according to ACMG classification appearing with a frequency of <1:25.000 in the GnomAD database and present in ≥ 7 affected individuals.[11]

Figure 2


A
1.0
0.8
0.6
0.4
0.2
0.0
Event-free survival
p = 0.30
Index-patients wit sporadic DCM
Index-patients with familial DCM
0
10
20
30
40
50
60
Age in years
No. at risk
Familial DCM 35 34 32 29 24 13 6
Sporadic DCM 74 69 65 63 48 26 9


B
1.0
0.8
0.6
0.4
0.2
0.0
Event-free survival
p < 0.001
Symptomatic relatives with DCM
Asymptomatic relatives with DCM
0
10
20
30
40
50
60
Age in years
No. at risk
Symptomatic 57 55 52 45 36 23 15
Asymptomatic 38 38 34 25 19 10 4

Event-free Survival of (A) Index-patients and (B) Relatives With DCM

(A) There was no difference in the event-free survival between index-patients with familial and sporadic DCM when including sudden cardiac death, ventricular arrhythmias, HTx and all-cause mortality in the analyses.

(B) Symptomatic relatives with DCM at diagnosis had a significantly shorter event-free survival rate compared to asymptomatic relatives with DCM at diagnosis, which was likely to be explained by the fact that symptomatic relatives were older at diagnosis and more severely affected than asymptomatic relatives. This finding also indicated that asymptomatic individuals with DCM may have benefited from family screening by being diagnosed and treated early in the course of the disease before they developed severe LV impairment and symptoms.

The Kaplan-Meier curves are truncated at age 62 since the sample size drops to <10% of the original sample size from the age of 62. The non-truncated Kaplan-Meier curves are provided in Supplemental Figure 1, Supplementary Materials.

Table 1 Clinical Characteristics of Index-patients With Familial or Sporadic DCM

Mean ± SD, median (IQR) or number (%)	Total n=109	Familial DCM n=35	Sporadic DCM n=74	p
Index-patients (%)	109 (100)	35/109 (32)	74/109 (68)	
Male sex (%)	76 (70)	24 (69)	52 (70)	0.85
Age at diagnosis in years ± SD	38.8 ± 15.7	39.0 ± 14.9	38.7 ± 16.2	0.92
Symptoms and echocardiography				
NYHA class III-IV at diagnosis (%)	66 (61)	21 (60)	45 (61)	0.93
LVEDd in mm ± SD at diagnosis	69 ± 12	69 ± 10	68 ± 13	0.77
LVEF in % ± SD at diagnosis	22 ± 10	25 ± 11	20 ± 9	0.02
Treatment				
ACE-I/AT-II receptor blocker during follow-up (%)	98 (90)	32 (91)	66 (89)	1.00
Beta-blockers during follow-up (%)	84 (77)	27 (77)	57 (77)	0.99
ICD + CRT-ICD (%)	20+23 (39)	7+7 (40)	13+16 (39)	0.94
CRT (%)	15 (14)	2 (6)	13 (18)	0.14
Follow-up				
Duration of follow-up in months (IQR)*	60 (25 – 106)	71 (40 – 129)	58 (18 – 88)	0.19
Atrial fibrillation (%)	35 (32)	15 (43)	20 (27)	0.10
Stroke or TIA (%)	6 (6)	2 (6)	4 (5)	1.00
HTx and death from heart failure				
(a) Familial DCM				
HTx index-patient without HTx-relatives + non-HTx index-patient with ≥ 1 HTx-relatives (%)	-	16+6 (63)	-	-
(b) Sporadic DCM				
HTx index-patients with sporadic DCM† (%)	-	-	47 (64)	-
Comparison of HTx in familial and sporadic DCM (%)	69 (63)	22 (63)	47 (64)	0.95
Age at HTx ± SD	42.4 ± 15.3	39.6 ± 18.8	43.4 ± 14.0	0.40
Arrhythmias and sudden cardiac death				
SCD, sustained VT or VF (%)	23 (21)	10 (29)	13 (18)	0.19
Age at SCD, sustained VT or VF ±SD	46.4 ± 11.7	43.7 ± 9.7	48.5 ± 13.1	0.34

*The follow-up period was from the time of diagnosis to the date of most recent follow-up. †One index-patient with sporadic DCM died from end-stage heart failure before receiving a cardiac transplant. ACE-I = angiotensin converting enzyme inhibitor; AT-II = angiotensinogen II; CRT = cardiac resynchronization therapy; HTx= heart transplantation; ICD = implantable cardioverter defibrillator; IQR = interquartile range; LVEDd= left ventricular end-diastolic diameter; LVEF= left ventricular ejection fraction; NA= not applicable or not available; NYHA= New York Heart Association; SCD = sudden cardiac death; SD = standard deviation; TIA = transient cerebral attack; VF = ventricular fibrillation; VT = ventricular tachycardia.

Table 2 Clinical Characteristics of Relatives With DCM

Mean ± SD, median (IQR) or number (%)	Total n=95	Symptoms at diagnosis n=57	No symptoms at diagnosis n=38	p
Relatives with DCM (%)	95 (100)	57/95 (60)	38/95 (40)	-
Male sex (%)	56 (59)	33 (58)	23 (61)	0.80
Age at diagnosis in years ± SD	38.7 ± 16.6	41.8 ± 16.3	33.9 ± 16.2	0.023
SCD or cardiac arrest as initial manifestation (%)	14 (15)	14 (25)	-	-
LVEDd in mm ± SD at diagnosis*	63 ± 8	66 ± 9	60 ± 6	0.002
LVEF in % ± SD at diagnosis*	32 ± 11	26 ± 11	38 ± 8	<0.001
Treatment				
ACE-I/AT-II receptor blocker during follow-up (%) †	56/69 (81)	23/31 (74)	33 (87)	0.18
Beta-blockers during follow-up (%) †	40/69 (58)	20/31 (65)	20 (53)	0.32
ICD + CRT-ICD (%)	16+7/83 (28)	6+6/45 (27)	10+1 (29)	0.82
Follow-up				
Duration of follow-up in months (IQR)‡	54 (3 – 104)	29 (0 – 98)	61 (24 – 104)	0.31
Atrial fibrillation (%)	12/83 (14)	8/45 (18)	4 (11)	0.35
HTx (%)	10/83 (12)	8/45 (18)	2 (5)	0.10
Age at HTx ±SD	31.0 ± 14.4	33.3 ± 15.3	21.7 ± 4.2	0.34
SCD, sustained VT or VF (%)	22 (23)	21 (37)	1 (3)	<0.001
Age at SCD, sustained VT or VF ± SD	37.5 ± 16.2	37.6 ± 16.5	35.1	-
Death among non-HTx, non-SCD patients (%)	14/70 (19)	14/34 (41)	0/36 (0)	<0.001
Age at death of non-HTx, non-SCD patients ± SD	54.7 ± 13.0	54.7 ± 13.0	-	-

*Echocardiography was available in 72 individuals with DCM. †Data on pharmacological treatment was available in 69 individuals. ‡The follow-up period was from the time of diagnosis to the date of most recent follow-up. Twelve relatives who died suddenly as initial manifestation of disease were excluded. Abbreviations: ACE-I = angiotensin converting enzyme inhibitor; AT-II = angiotensinogen II; CRT = cardiac resynchronization therapy; HTx= heart transplantation; ICD = implantable cardioverter defibrillator; IQR = interquartile range; LVEDd= left ventricular end-diastolic diameter; LVEF= left ventricular ejection fraction; NYHA= New York Heart Association; SCD = sudden cardiac death; SD = standard deviation; VF = ventricular fibrillation; VT = ventricular tachycardia.

Table 3: High-Confidence Pathogenic DNA Sequence Variants[†] Suitable for Genetic Testing Identified Following Genetic Investigations of 76 Recognised and Likely DCM Genes

Gene	Genomic position	Amino acid / Nucleotide change	AF in gnomAD	ACMG classification	Number of families[§] (affected/healthy individuals in the present study and affected individuals from *previous* studies)	References
LMNA	156105716	p.Arg321*/c.961C>T	-	P	2 (7/9/*4*)	[20, 21]
LMNA	156106742	p.Arg471Cys/c.1411C>T	3/251152	P	1 (2/1/*5*[‡])	[22‡]
LMNA	156106743	p.Arg471His/c.1412G>A	-	P	1 (9/4/*8*)	[23, 24]
LMNA	156106957	p.Trp514*/c.1542G>A	-	P	1 (4/4/*5*)	[24]
MYH7	23896496	p.Lys637Glu/c.1909A>G	-	P	1 (7/2/*6*)	[25]
RBM20	112572056	p.Arg634Gln/c.1901G>A	-	P	1 (2/6/*10*)	[26-28]
RBM20	112572062	p.Arg636His/c.1907G>A	-	P	1 (3/2/*10*)	[4, 27-29]
RBM20	112572061	p.Arg636Ser/c.1906C>A	-	P	4 (25/9/*9*)	[26, 28]
RBM20	112581114	p.Glu913Lys/c.2737G>A	-	P	1 (8/1/*8*)	[28, 30]
TNNT2	201333469	p.Arg139His/c.416G>A	-	P	1 (6/3/*1*)	[31]
TNNT2	201332476	p.Arg173Gln/c.518G>A	-	P	1 (1/0/*10*)	[32-34]
TPM1	63353128	p.Leu227Phe/c.679C>T	-	P	1 (8/0/*0*)	Novel
TTN	179398832	p.Trp34170*/c.102510G>A	-	P	2 (8/1/*0*)	Novel
Total					18 (90/42/*76*)	

[†] Pathogenic/likely pathogenic sequence variants according to ACMG classification appearing with a frequency of <1:25.000 in the GnomAD database and present in ≥ 7 affected individuals.[11]

[‡]Identified in one additional family with four affected mutation carriers (unpublished data). AF = allele frequency; P = pathogenic. [§]Number of families carrying the sequence variant and in brackets: first row = number of affected carriers in the present study; second row = healthy carriers in the present study; third row (*underlined and in italic*) = affected carriers from previous studies.

Table 4 Clinical Characteristics of DCM Patients Carrying High-Confidence Pathogenic DNA Sequence Variants* Suitable for Genetic Testing in *RBM20* and *LMNA*

Mean ± SD, median (IQR) or number (%)	Total	*RBM20*	*LMNA*	p
Number of index-patients (%)	12/12 (100)	7/12 (58)	5/12 (42)	
Penetrance (%)	60/96 (63)	38/56 (68)	22/40 (55)	0.20
Male sex (%)	34/60 (57)	22/38 (58)	12/22 (55)	0.80
Age at diagnosis ± SD	39.3 ± 14.0	35.4 ± 13.6	45.9 ± 12.3	0.004
Age at most recent follow-up of healthy carriers ± SD	30.7 ± 14.8	34.0 ± 15.8	27.4 ± 13.4	0.19
Symptoms and Echocardiography				
NYHA class III-IV at diagnosis (%)†	13/54 (24)	9/35 (26)	4/19 (21)	0.70
LVEDd in mm ± SD at diagnosis‡	64 ± 8	65 ± 8	60 ± 7	0.09
LVEF in % ± SD at diagnosis‡	32 ± 13	31 ± 12	32 ± 13	0.85
Treatment				
ACE-I/AT-II receptor blockers during follow-up (%)§	41/48 (85)	31/35 (89)	10/13 (77)	0.31
Beta-blockers during follow-up (%)§	35/48 (73)	25/35 (71)	10/13 (77)	0.70
ICD + CRT-ICD (%)	18+11/54 (54)	13+6/35 (50)	5+5/19 (45)	0.91
Follow-up				
Duration of follow-up in months (IQR)#	64 (14 – 122)	79 (46 – 161)	56 (3 – 88)	0.04
Atrial fibrillation (%)	16/54 (30)	8/35 (23)	8/19 (42)	0.14
HTx (%)	11/54 (20)	7/35 (20)	4/19 (21)	0.93
Age at HTx ± SD	38.8 ± 15.8	34.9 ± 15.4	45.5 ± 16.4	0.31
SCD, sustained VT or VF (%)	19/60 (32)	13/38 (34)	6/22 (27)	0.58
Age at SCD, sustained VT or VF ± SD	42.0 ± 11.2	42.5 ± 11.6	40.8 ± 11.2	0.77
Death among non-HTx, non-SCD patients (%)	9/41 (22)	2/27 (7)	7/14 (50)	0.002
Age at death of non-HTx, non-SCD patients ± SD	58.2 ± 10.9	63.2 ± 13.8	56.5 ± 10.7	0.43

*Pathogenic/likely pathogenic sequence variants according to ACMG classification appearing with a frequency of <1:25.000 in the GnomAD database and present in ≥ 7 affected individuals.[11]

†Information about NYHA class was available for 54 individuals. ‡Echocardiography was available in 47 individuals with DCM. §Data on pharmacological treatment was available in 48 individuals. #The follow-up period was from the

time of diagnosis to the date of most recent follow-up. Six individuals who died suddenly as initial manifestation of disease were excluded from these calculations.

ACE-I = angiotensin converting enzyme inhibitor; AT-II = angiotensinogen II; CRT = cardiac resynchronization therapy; HTx= heart transplantation; ICD = implantable cardioverter defibrillator; IQR = interquartile range; LVEDd= left ventricular end-diastolic diameter; LVEF= left ventricular ejection fraction; NYHA= New York Heart Association; SCD = sudden cardiac death; SD = standard deviation; VF = ventricular fibrillation; VT = ventricular tachycardia.