Clinical utility of genetic testing in patients with dilated cardiomyopathy

Utilidad clínica del estudio genético en pacientes con miocardiopatía dilatada

Maria Luisa Peña-Peña

Servicio de Cardiología, Hospital Universitario Virgen del Rocío, Sevilla, Spain

Universidad de A Coruña (UDC), A Coruña, Spain

Juan Pablo Ochoa

Instituto de Investigación Biomédica de A Coruña (INIBIC), Comité Científico, Health in Code, A Coruña, Spain

Roberto Barriales-Villa

Servicio de Cardiología, Complejo Hospitalario Universitario A Coruña (CHUAC), Universidad de A Coruña (UDC), Instituto de Investigación Biomédica de A Coruña (INIBIC), A Coruña, Spain

Centro de Investigación Biomédica en Red de Enfermedades Cardiovasculares (CIBERCV), Instituto de Salud Carlos III, Madrid, Spain

Marcos Cicerchia

Instituto de Investigación Biomédica de A Coruña (INIBIC), Comité Científico, Health in Code, A Coruña, Spain

Julián Palomino-Doza

Centro de Investigación Biomédica en Red de Enfermedades Cardiovasculares (CIBERCV), Instituto de Salud Carlos III, Madrid, Spain

Servicio de Cardiología, Hospital Universitario 12 de Octubre, Madrid, Spain

Joel Salazar-Mendiguchia

Instituto de Investigación Biomédica de A Coruña (INIBIC), Comité Científico, Health in Code, A Coruña, Spain

Arsonval Lamounier

Juan Pablo Trujillo

Instituto de Investigación Biomédica de A Coruña (INIBIC), Comité Científico, Health in Code, A Coruña, Spain

Diego Garcia-Giustiniani

Instituto de Investigación Biomédica de A Coruña (INIBIC), Comité Científico, Health in Code, A Coruña, Spain

Xusto Fernandez

Instituto de Investigación Biomédica de A Coruña (INIBIC), Comité Científico, Health in Code, A Coruña, Spain

Martin Ortiz-Genga

Instituto de Investigación Biomédica de A Coruña (INIBIC), Comité Científico, Health in Code, A Coruña, Spain

Lorenzo Monserrat

Instituto de Investigación Biomédica de A Coruña (INIBIC), Comité Científico, Health in Code, A Coruña, Spain

Maria Generosa Crespo-Leiro

Servicio de Cardiología, Complejo Hospitalario Universitario A Coruña (CHUAC), Universidad de A Coruña (UDC), Instituto de Investigación Biomédica de A Coruña (INIBIC), A Coruña, Spain

Centro de Investigación Biomédica en Red de Enfermedades Cardiovasculares (CIBERCV), Instituto de Salud Carlos III, Madrid, Spain

Corresponding author. E-mail address: marialuisacardio@gmail.com (M.L. Peña-Peña).

Abstract

Introduction and objectives. Dilated cardiomyopathy (DCM) is the most frequent cause of heart transplantation. The prevalence of familial disease can reach 50%. Our objective was to describe the genetic basis of DCM in a cohort with a high proportion of transplanted patients.

Methods. We included patients with DCM and genetic testing performed using next-generation sequencing (NGS) that included at least 80 genes. Clinical data, family history and genetic results were retrospectively analysed. When possible, assessment of first-degree relatives was carried out.

Results. Eighty-seven DCM patients and 308 relatives from 70 families were evaluated. Clinical prevalence of familial disease was 37% (32 patients). Forty-four percent of patients (38 patients) had required heart transplantation. A relevant variant was found in 43 patients (49%), 25 patients (29%) carried variants of unknown significance and in 19 patients (22%) the study was negative. Most genetic variants were found in sarcomeric genes and the yield of genetic testing was higher in patients with familial DCM.

Conclusions. The yield of genetic testing in our DCM cohort was high, reaching 69% in familial cases. Mutational spectrum was heterogeneous and the identification of the specific aetiology of the disease often provided prognostic information.

Resumen

Introducción y objetivos. La miocardiopatía dilatada (MCD) es la causa más frecuente de trasplante cardiaco. Se considera que es familiar hasta en el 50% de los casos. Nuestro objetivo es describir los resultados genéticos obtenidos en una cohorte de pacientes con MCD, de los cuales una elevada proporción había acabado en trasplante cardiaco.

Métodos. Se incluyeron pacientes con MCD a los que se realizó *next-generation sequencing* (NGS, «secuenciación de nueva generación») de al menos 80 genes relacionados con la enfermedad. Se analizaron retrospectivamente los datos clínicos de los pacientes, la historia familiar y los resultados del estudio genético. En los casos en los que fue posible, se realizó una evaluación de sus familiares de primer grado.

Resultados. Fueron evaluados 87 pacientes con MCD y 308 familiares de 70 familias distintas. La prevalencia clínica de enfermedad familiar fue del 37% (32 pacientes) y el 44% (38 pacientes) habían precisado un trasplante cardiaco. En 43 pacientes (49%) se encontró al menos una variante relevante, en 25 pacientes (29%) se identificaron variantes de significado incierto y en 19 pacientes (22%) el estudio fue negativo. La mayoría de las mutaciones se encontraron en genes sarcoméricos y la rentabilidad del estudio fue mayor en los pacientes con MCD familiar.

Conclusiones. El estudio genético NGS en nuestra población de pacientes con MCD tuvo una elevada rentabilidad, alcanzando el 69% en los casos familiares. El espectro mutacional fue heterogéneo y con frecuencia la identificación de la etiología específica de la enfermedad aportó información pronóstica.

Keywords

Dilated cardiomyopathy; Heart transplant; Genetic testing; Mutation

Palabras clave

Miocardiopatía dilatada; Trasplante cardiaco; Estudio genético; Mutación

Introduction

Dilated cardiomyopathy (DCM) is defined as ventricular or biventricular systolic dilation and dysfunction in the absence of abnormal loading conditions or coronary artery disease.[1] It has a high mortality and is the most common cause of heart transplantation.[2] The disease is considered to be familial in up to 50% of cases and in recent years more than 90 genes have been identified as being involved. Of these genes, the most commonly identified is the *TTN gene*, in both sporadic and familial cases.[3] Recently, a high cost-effectiveness of genetic testing has been observed in patients with DCM undergoing heart transplantation, higher than in other cardiomyopathies for which the study is generally recommended.[4]

The aim of our paper is to describe the genetic results obtained in a large cohort of patients with DCM, many of whom underwent heart transplantation for treatment of their disease.

Methods

Eighty-seven consecutive patients with DCM evaluated in a specialist practice between February 2014 and December 2016 were included and underwent *next-generation sequencing* (NGS), evaluating at least 80 disease-related genes (Table 1). Patients with significant coronary artery disease (stenosis greater than 50%), severe valve disease, severe hypertension, or myocarditis were excluded. DCM was considered familial when there was more than one affected member and in cases with a history of sudden death in first-degree relatives under 35 years of age.[5] The clinical data of the patients, the family history and the results of their genetic study were retrospectively analysed. All first-degree relatives were offered an in-office evaluation by physical examination, electrocardiogram, and echocardiogram, as well as a targeted genetic testing where necessary. The study protocol was approved by the A Coruña-Ferrol Research Ethics Committee.

The genetic study included the analysis of all coding exons and flanking intronic regions. Relevant variants identified according to the patient's phenotype were confirmed by Sanger sequencing in both directions, which was also used to resequence low coverage regions. To evaluate the pathogenicity of the identified variants, an algorithm based on the modified criteria of the American Society for Medical Genetics and Genomic

Medicine was used.[6] Where possible, the cosegregation of the variants with the familial disease was evaluated. The final classification of each variant was made by consensus of 2 cardiologists with expertise in the interpretation of genetic variants. The variants were finally classified into pathogenic, possibly pathogenic, and variants of uncertain significance.

Results

Eighty-seven DCM patients and 308 relatives from 70 different families participated. The clinical and family characteristics of the evaluated patients are detailed in Table 2. The disease was classified as familial in 37% of the cases. The genealogical trees of the most informative families are shown in Fig. 1. 44% of the patients were transplanted (Tx). The mean age at diagnosis was 43 ± 21 years (17 months-74 years) and 75% were male. The mean diastolic diameter was 62.9 ± 20.8 mm and the mean ejection fraction (EF) was 28%. There were no significant clinical or echocardiographic differences between Tx and non-Tx, except in EF, which was lower in Tx (21 ± 23 versus 34 ± 12, $p < 0.05$).

The cosegregation of the identified variants could be assessed in 17 of the families analysed. The family study supported the pathogenicity of 14 of the variants considered pathogenic or possibly pathogenic. In 3 of the cases (families 1, 12 and 35), the family study questioned the pathogenicity of the variants identified, as some affected family members were not carriers; therefore, these were finally classified as of uncertain significance.

Table 3, Table 4, describe the characteristics of the identified mutations that were considered pathogenic or possibly pathogenic after the family study. In 43 patients (49%), at least one relevant variant was found, identifying 23 pathogenic variants in 22 patients and 26 possibly pathogenic variants in 23 patients. Five patients (6%) had more than one variant identified that could explain the disease. In 25 patients (29%) only variants of uncertain significance were identified and in 19 patients (22%) the genetic study was negative.

Mutations were found most commonly in sarcomeric genes. *TTN* gene truncating variants explained 14% of the cases (12 patients). In 7 of the patients (8%) variants were found in *MYH7* and in 5 patients (6%) variants were identified in *MYBPC3,* although in 2 of them a second associated variant was identified. Variants were also identified in *TPM1* and

TNNI3 (one patient each). Four patients (5%) had variants in *LMNA* and a mutation carrier was identified in *DMD* and another in *DES.* Mutations in desmosomal genes were found in 10 patients (12%), the majority in *DSG2* and *DSP.* Other genes identified with lower prevalence were *FLNC* and *RBM20.*

The cost-effectiveness of the study, understood as the identification of a pathogenic or possibly pathogenic variant, was higher in patients with familial versus non-familial DCM (69% vs. 42%, $p < 0.05$). A higher cost-effectiveness of genetic testing was not observed in Tx versus non-Tx patients (47 versus 55%, $p = 0.47$).

Discussion

Our study shows the cost-effectiveness of genetic testing using NGS in a cohort of patients with DCM. The mutational spectrum is heterogeneous, and, in some cases, more than one variant has been identified as being involved in the development of the disease. The probability of identifying a relevant variant increases significantly if the disease is familial.

Before genetics was applied to the study of this disease, the estimated prevalence of familial DCM was approximately 30% in papers that performed a systematic evaluation.[7] Subsequently, with the development of NGS, more than 90 genes have been associated with the development of this disease, although the evidence of this association in many cases is not consistent due to lack of clinical data and limited cosegregation studies in the families studied. The disease is now considered to be familial in up to 50% of cases.[8] Our study found a clinical prevalence of familial disease of 37% and a cost-effectiveness of genetic testing of 49%, which increases to 69% when there is a family history, data that are consistent with those previously described in the medical literature, taking into account that this is a selected population of DCM where patients with severe phenotypes were included.

Regarding the population of Tx patients, a study carried out by our group in 2002 with 43 DCM Tx patients found a high prevalence of familial disease, with more than 50% of cases susceptible to having a genetic condition.[9] A recent study that performed NGS in 52 DCM Tx patients, in addition to a complete analysis of relatives, identified the molecular cause of the disease in 40% of the patients evaluated, taking into account only the data from genetic testing, and in up to 73% of the patients after an exhaustive

evaluation of the relatives.[4] Thirty-eight DCM Tx patients were included in our population, in which the cost-effectiveness of genetic testing was 47%. In most cases, a cosegregation study could not be performed, because only 8 of them had other affected living relatives and some could not be evaluated.

Most of the mutations identified were found in sarcomeric genes and, specifically, in the *TTN gene.* Different studies show that this gene is the most commonly affected in DCM, assuming the molecular cause of 14–25% of cases.[3,10] In our study, *TTN* truncating variants were identified in 22% (7/32) of familial cases and only in 9% of sporadic cases (5/55). Initial studies of patients with DCM and truncating *TTN* variants found no prognostic differences between carriers of these variants and non-carriers.[3] These types of mutations may have a better prognosis, compared to cases with mutations in *LMNA* or a negative genetic study.[11] However, our population shows a high incidence of episodes from the age of 30 onwards, mainly in male relatives. Of the 12 families with truncating *TTN* variants, at least 5 had a history of sudden death and/or heart failure.

Mutations in genes encoding sarcomere proteins have been primarily associated with hypertrophic cardiomyopathy (HCM), although most are also involved in DCM. An overlap may commonly occur between the two phenotypes and also with restrictive and non-compaction cardiomyopathy. Prognostic differences have been observed in *MYH7* variants based on their location in the protein. Variants located in the converting region (in our series, Ile724Thr) have been associated with a high prevalence of episodes.[12] Mutations located in the actin-binding cleft (such as Val338Met and Glu344Lys) are often associated with early phenotypes.[13] Variants that are located in the tail of the protein (in our study, Ser1102Thr, Lys910Arg, Ala990Thr and Arg1697Trp) can produce DCM without prior HCM data.[10] *MYBPC3* variants are rarer, and although in some series they are described as a common cause of disease,[14] its pathogenicity is often uncertain. In some cases, these types of variants could require an additional factor to cause disease, as in 2 of our cases, which had a second associated mutation. As for *TPM1* variants, those in the central region of the flexible C-terminal end (in our case, Asp230Asn) have been associated with DCM and carriers are often described at early ages, even in paediatric age.[15]

LMNA mutations are associated with DCM with a high risk of sudden death and ventricular conduction disorders and arrhythmias characteristically precede ventricular

dysfunction.[16] Four factors have been identified that independently increase the risk of arrhythmias in carriers: the presence of nonsustained ventricular tachycardia, EF < 45%, male sex and mutations that are not *missense*.[17] In our series, carriers of mutations in this gene (all of them *missense*) had severe phenotypes and episodes of sudden death and/or transplantation are described in the 4 families. It is noteworthy that 2 of the family members with events had pacemakers, highlighting the need to protect these patients with the implantation of a cardioverter-defibrillator.[18] Among the mutations in cytoskeletal proteins, it is interesting to highlight the mutation identified in *DES* (Arg275Gly) in a patient who started with sudden death and mild ventricular dysfunction. Variants in this gene have been associated with a restrictive phenotype with conduction disorders and progressive skeletal myopathy, although there are pathogenic variants that can manifest as DCM and arrhythmogenic cardiomyopathy (ACM).[19]

Desmosomal genes should be considered in the genetic diagnosis of DCM, since although they are primarily related to the development of ACM, there may be left-dominant or biventricular forms that are indistinguishable from DCM. The most commonly related genes are *DSP* and *PKP2*, and a higher incidence of ventricular arrhythmias and a higher risk of sudden death have been described, regardless of the EF.[20,21] Anyway, the interpretation of variants, especially *missense*, in these genes must be careful since penetrance could be low and not even a sufficient cause of disease.

In our population we identified 2 variants in *RBM20* (Arg711Cys and Gln176Glu). We have information about the first of them, which is located in a relevant functional region (junction site between the Arg/Ser-rich region and zinc-finger domain). In this family, the proband required heart transplantation at an early age, as has been observed for close mutations that have also been associated with a poor prognosis.[22] Regarding the variant identified in *FLNC* (Pro963Argfs*26), it has already been described by our group in a previous study where the association of this type of variants with an overlapping left ventricle DCM and ACM phenotype, with high penetrance was observed.[23] An almost exclusive left-sided involvement with extensive intramyocardial fibrosis, high frequency of ventricular arrhythmias and absence of skeletal myopathy is characteristic.

More than one relevant variant was identified that could explain the disease in 6% of the patients. This has been described in recent studies on the genetics of DCM and could explain the differences in penetrance and phenotypic expression observed in some

families.[14] Our study did not show relevant phenotypic differences between these patients and the rest of the population.

Cosegregation studies were limited in our study due to a low participation in the family study. Although an average of 4.4 ± 2.8 relatives per patient were assessed, there were 17 families who did not participate in the study. There was less familial disease in those who declined to participate, although in some cases a history was evident. The inclusion of the most severe cases limits the generalizability of the results to all patients with DCM. On the other hand, a high prevalence of healthy carriers was observed, which may be due to the fact that most were identified at an early age in the context of the family study. In any case, the high cost-effectiveness of genetic testing, especially in cases of familial disease, highlights the importance of an adequate clinical and genetic evaluation of first-degree relatives in patients with DCM.

Conclusions

Genetic testing using NGS in our population DCM patients had a cost-effectiveness of 49%, increasing to 69% in familial cases. The mutational spectrum was heterogeneous and the identification of the specific aetiology of the disease often provided prognostic information.

Conflict of interests

Juan Pablo Ochoa, Marcos Cicerchia, Joel Salazar-Mendiguchia, Arsonval Lamounier, Diego Garcia-Giustiniani, Xusto Fernandez and Martin Ortiz-Genga work for Health in Code SL. Lorenzo Monserrat is CEO of Health in Code SL.

References

1. Elliott P, Andersson B, Arbustini E, Bilinska Z, Cecchi F, Charron P, et al. Classification of the cardiomyopathies: a position statement from the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. Eur Heart J. 2008;29:270–6.
2. Lund LH, Edwards LB, Kucheryavaya AY, Benden C, Dipchand AI, Goldfarb S, et al. The Registry of the International Society for Heart and Lung Transplantation: thirty-

second official adult heart transplantation report—2015; Focustheme: early graft failure. J Heart Lung Transplant. 2015;34:1244–54.

3. Herman DS, Lam L, Taylor MR, Wang L, Teekakirikul P, Christodoulou D, et al. Truncations of titin causing dilated cardiomyopathy. N Engl J Med. 2012;366:619–28.
4. Cuenca S, Ruiz-Cano MJ, Gimeno-Blanes JR, Jurado A, Salas C, Gomez-Diaz I, et al. Genetic basis of familial dilated cardiomyopathy patients undergoing heart transplantation. J Heart Lung Transplant. 2016;35:625–35.
5. Mestroni L, Maisch B, McKenna WJ, Schwartz K, Charron P, Rocco C, et al. Guidelines for the study of familial dilated cardiomyopathies. Collaborative Research Group of the European Human and Capital Mobility Project on Familial Dilated Cardiomyopathy. Eur Heart J. 1999;20:93–102.
6. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405–24.
7. Castro-Beiras A, Monserrat L, Hermida M. Miocardiopatía dilatada familiar: situación actual y beneficios clínicos de la investigación básica. Rev Esp Cardiol. 2003;56:7–12.
8. Burkett EL, Hershberger RE. Clinical and genetic issues in familial dilated cardiomyopathy. J Am Coll Cardiol. 2005;45:969–81.
9. Monserrat L, Hermida M, Bouzas B, Mosquera I, Mahon N, Peteiro J, et al. Familial dilated cardiomyopathy in patients transplanted for idiopathic dilated cardiomyopathy. Rev Esp Cardiol. 2002;55:725–32.
10. Pugh TJ, Kelly MA, Gowrisankar S, Hynes E, Seidman MA, Baxter SM, et al. The landscape of genetic variation in dilated cardiomyopathy as surveyed by clinical DNA sequencing. Genet Med. 2014;16:601–8.
11. Jansweijer JA, Nieuwhof K, Russo F, Hoorntje ET, Jongbloed JD, Lekanne Deprez RH, et al. Truncating titin mutations are associated with a mild and treatable form of dilated cardiomyopathy. Eur J Heart Fail. 2016;19:512–21.
12. García-Giustiniani D, Arad M, Ortíz-Genga M, Barriales-Villa R, Fernández X, Rodríguez-García I, et al. Phenotype and prognostic correlations of the converter region mutations affecting the myosin heavy chain. Heart. 2015;101:1047–53.
13. Ackerman MJ, VanDriest SL, Ommen SR, Will ML, Nishimura RA, Tajik AJ, et al. Prevalence and age-dependence of malignant mutations in the β-myosin heavy chain and troponin T genes in hypertrophic cardiomyopathy comprehensive outpatient perspective. J Mol Cell Cardiol. 2002;39:2042–8.

14. Haas J, Frese KS, Peil B, Kloos W, Keller A, Nietsch R, et al. Atlas of the clinical genetics of human dilated cardiomyopathy. Eur Heart J. 2015;36:1123–35.
15. Lakdawala NK, Dellefave L, Redwood CS, Sparks E, Cirino AL, Depalma S, et al. Familial dilated cardiomyopathy caused by an alpha-tropomyosin mutation: the distinctive natural history of sarcomeric dilated cardiomyopathy. J Am Coll Cardiol. 2010;55:320–9.
16. Hermida-Prieto M, Monserrat L, Castro-Beiras A, Laredo R, Soler R, Peteiro J, et al. Familial dilated cardiomyopathy and isolated left ventricular noncompaction associated with lamin A/C gene mutations. Am J Cardiol. 2004;94:50–4.
17. Van Rijsingen IA, Arbustini E, Elliott P, Mogensen J, Hermans-van Ast JF, van der Kooi AJ, et al. Risk factors for malignant ventricular arrhythmias in lamin A/C mutation carriers: a European cohort study. J Am Coll Cardiol. 2012;59:493–500.
18. Anselme F, Moubarak G, Savouré A, Godin B, Borz B, Drouin-Garraud V, et al. Implantable cardioverter-defibrillators in lamin A/C mutation carriers with cardiac conduction disorders. Heart Rhythm. 2013;10:1492–8.
19. Bermúdez-Jiménez FJ, Carriel V, Brodehl A, Alaminos M, Campos A, Schirmer I, et al. Novel desmin mutation p.Glu401Asp impairs filament formation, disrupts cell membrane integrity, and causes severe arrhythmogenic left ventricular cardiomyopathy/dysplasia. Circulation. 2018;137:1595–610.
20. Elliott P, O'Mahony C, Syrris P, Evans A, Rivera Sorensen C, Sheppard MN, et al. Prevalence of desmosomal protein gene mutations in patients with dilated cardiomyopathy. Circ Cardiovasc Genet. 2010;3:314–22.
21. Spezzacatene A, Sinagra G, Merlo M, Barbati G, Graw SL, Brun F, et al. Familial Cardiomyopathy Registry. Arrhythmogenic phenotype in dilated cardiomyopathy: natural history and predictors of life-threatening arrhythmias. J Am Heart Assoc. 2015;4:e002149.
22. Li D, Morales A, Gonzalez-Quintana J, Norton N, Siegfried JD, Hofmeyer M, et al. Identification of novel mutations in RBM20 in patients with dilated cardiomyopathy. Clin Transl Sci. 2010;3:90–7.
23. Ortiz-Genga MF, Cuenca S, Dal Ferro M, Zorio E, Salgado-Aranda R, Climent V, et al. Truncating FLNC mutations are associated with high-risk dilated and arrhythmogenic cardiomyopathies. J Am Coll Cardiol. 2016;68:2440–51.

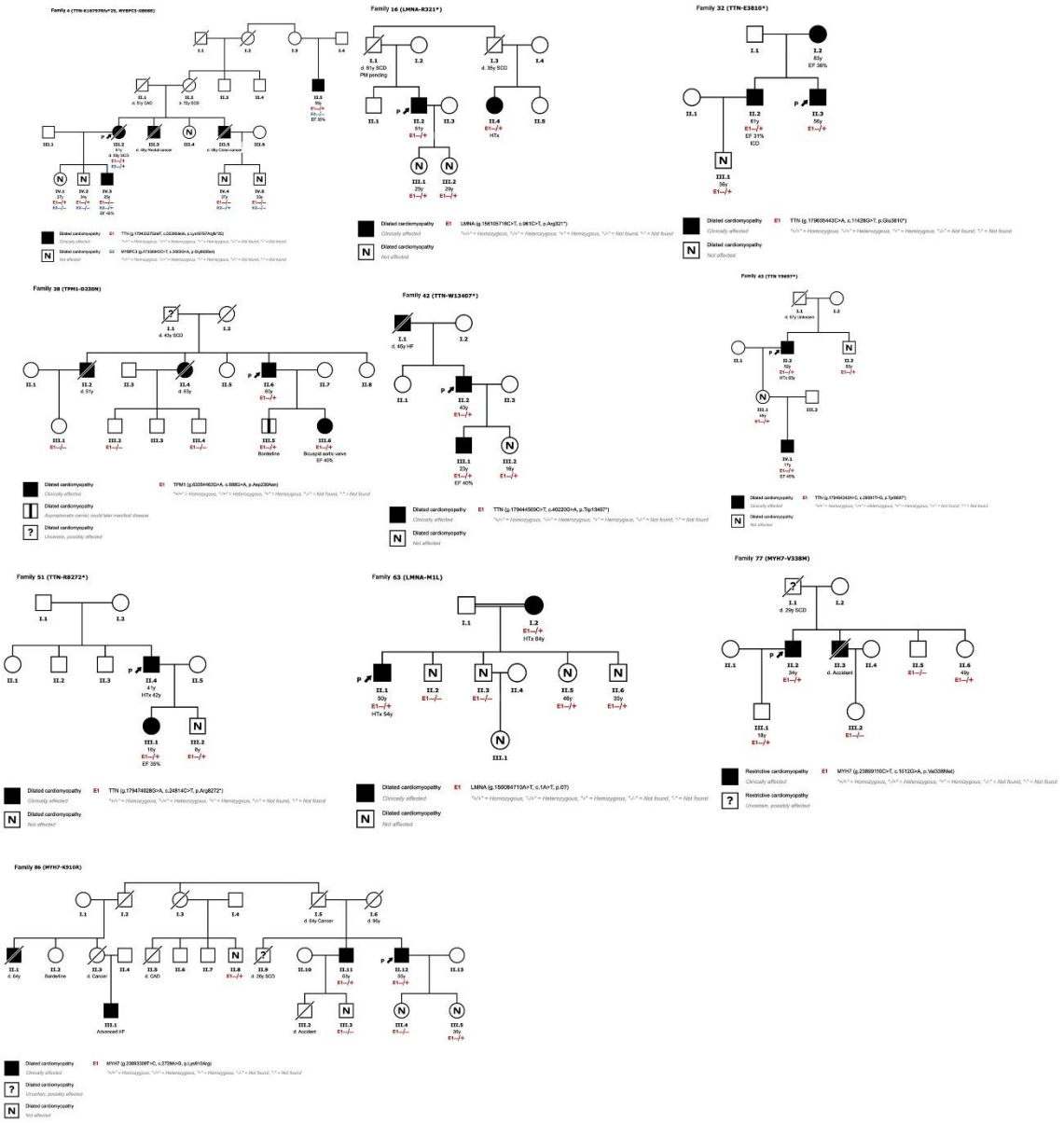

Fig. 1. Family trees of the most informative families with dilated cardiomyopathy in the study. Squares and circles indicate male and female individuals, respectively. Arrows indicate probands. Symbols with a slash indicate deceased family members. Solid black symbols are affected relatives and those with an "N" are non-affected. The double horizontal line indicates consanguinity. CAD: *coronary artery disease*; EF: *ejection fraction*; HF: *heart failure*; HTx: *heart transplant*; PM: *pacemaker*; SCD: *sudden cardiac death*.

Table 1. Main genes associated with dilated cardiomyopathy.

Gene	Protein	Priority
TTN	Titin	Priority
LMNA	Lamin A/C	Priority
DMD	Dystrophin	Priority
MYH7	Beta-myosin heavy chain	Priority
DSP	Desmoplakin	Priority
BAG3	BAG protein family chaperone activity regulator	Priority
FLNC	Filamin C	Priority
ACTC1	Actin	Priority
RBM20	RNA 20 binding protein	Priority
TNNT2	Troponin T	Priority
MYBPC3	Myosin-binding protein C	Priority
PKP2	Plakophilin	Priority
PLN	Phospholamban	Priority
DES	Demining	Priority
TNNI3	Troponin I	Priority
TNNC1	Troponin C	Priority
TPM1	Tropomyosin	Priority
TAZ	Tafazine	Priority

Table 1. Main genes associated with dilated cardiomyopathy.

Gene	Protein	Priority
ABCC9	ATP-binding cassette subfamily C member 9 gene	Secondary
ACTA1	Alpha actin 1	Secondary
ACTN2	Alpha actinin 2	Secondary
ALMS1	ALMS1 protein	Secondary
ANKRD1	Ankyrin repeat domain 1	Secondary
ANO5	Anoctamin 5	Secondary
CAV3	Caveolin 3	Secondary
CHRM2	Cholinergic Receptor Muscarinic 2	Secondary
COL7A1	Collagen Type VII Alpha 1 Chain	Secondary
CRYAB	Crystallin Alpha B	Secondary
CSRP3	Cysteine and Glycine Rich Protein 3	Secondary
DNAJC19	Mitochondrial inner membrane transporter	Secondary
DOLK	Dolichol kinase	Secondary
DSC2	Desmocollin	Secondary
DSG2	Desmoglein	Secondary

Table 1. Main genes associated with dilated cardiomyopathy.

Gene	Protein	Priority
EMD	Emerin	Secondary
EYA4	Eyes absent homolog 4	Secondary
FHL2	Four-and-a-half LIM domains protein	Secondary
FHOD3	Formin FH1/FH2 domain	Secondary
FKRP	Fukutin-related protein	Secondary
FKTN	Fukutin	Secondary
FOXD4	FOXD4 nuclear transcription factor	Secondary
GAA	Acid alpha-glucosidase	Secondary
GATA4	GATA4 transcription factor	Secondary
GATA6	GATA6 transcription factor	Secondary
GATAD1	GATAD1 protein	Secondary
GLB1	Beta-galactosidase	Secondary
HFE	Iron transport protein	Secondary
JUP	Plakoglobin	Secondary
LAMA2	Laminin alfa 2	Secondary
LAMA4	Laminin alpha 4	Secondary
LAMP2	Lysosomal Associated Membrane Protein 2	Secondary

Table 1. Main genes associated with dilated cardiomyopathy.

Gene	Protein	Priority
LDB3	LIM domain binding 3 protein	Secondary
MURC	Caveolae associated protein 4	Secondary
MYH6	Myosin heavy chain 6	Secondary
MYL2	Myosin regulatory light chain 2	Secondary
MYL3	Myosin light polypeptide 3	Secondary
MYOT	Myotilin	Secondary
MYPN	Myopalladin	Secondary
NEBL	Nebulette	Secondary
NEXN	Nexilin	Secondary
PRDM16	PRDM16 protein	Secondary
PSEN1	Presenilin 1	Secondary
PSEN2	Presenilin 2	Secondary
RAF1	RAF proto-oncogene serine/threonine-protein kinase	Secondary
RYR2	Ryanodine receptor	Secondary
SCN5A	Sodium Voltage-Gated Channel Alpha Subunit 5	Secondary
SDHA	Flavoprotein (FP) subunit of the mitochondrial respiratory chain	Secondary

Table 1. Main genes associated with dilated cardiomyopathy.

Gene	Protein	Priority
SGCD	Delta-sarcoglycan	Secondary
SLC22A5	SLC22A5 protein	Secondary
SPEG	Ser/Thr striated muscle enriched protein kinase	Secondary
SYNE1	Nesprin 1	Secondary
SYNE2	Nesprin 2	Secondary
TBX20	Transcription factor TBX20	Secondary
TCAP	Telethonin	Secondary
TMEM43	Transmembrane protein 43	Secondary
TMPO	Thymopoietin	Secondary
TOR1AIP1	Torsin 1A interacting protein 1	Secondary
TTR	Transthyretin	Secondary
TXNRD2	Thioredoxin reductase 2	Secondary
VCL	Vinculin	Secondary
XK	Membrane transport protein XK	Secondary
BRAF	Serine/threonine kinase BRAF protein	Candidate
DNM1L	Dynamin 1-like protein	Candidate
GATA5	GATA5 transcription factor	Candidate

Table 1. Main genes associated with dilated cardiomyopathy.

Gene	Protein	Priority
GLA	Alpha galactosidase A	Candidate
IDH2	IDH2 mitochondrial protein	Candidate
ILK	Integrin-linked kinase protein	Candidate
KCNJ2	Kir2.1 inward rectifier-type potassium channel subunit	Candidate
KCNJ8	Kir6.1 inward rectifier-type potassium channel subunit	Candidate
NKX2-5	Transcription factor NKX2-5	Candidate
OBSCN	Obscurin	Candidate
OPA3	Optic atrophy 3 protein	Candidate
PDLIM3	PDZ and LIM domain protein 3	Candidate
PTPN11	Tyrosine phosphatase 11 protein	Candidate
SGCA	Alpha-sarcoglycan	Candidate
SGCB	Beta-sarcoglycan	Candidate
TNNI3K	Serine/threonine-protein kinase TNNI3K	Candidate

Table 2. Clinical and family characteristics of the 87 genotyped patients with dilated cardiomyopathy.

Patient	Age	Sex	Tx (age)	Genetic variant	Classification of variants	Clinical data	Familial history	Relatives assessed	Relatives affected	Carrier relatives	Carriers with phenotype
1	50	M	No	LDB3-S203W	VUS	EDD 66 mm, EF 47%, 14 mm thickness. Alcohol (mild) SD at 53	Brother with DCM and SD at 67, sister with DCM and ICD Son SD at 42 (HCM autopsy)	16	2	3	0
2	43	M	Yes (47)	**LMNA-E124K** **MYH7-S1102T**	PPV PPV	EDD 57 mm, EF 23% Alcohol (moderate)	Mother died at 67 (cardiac disease, PM carrier)	6	0	4	0
3	48	M	Yes (51)	**DSP-R925P**	PPV	EDD 72 mm, EF 19%	Father with DCM lives at 87	3	1	0	0
4	51	F	No	**TTN-K16797Rfs*25** **MYBPC3-G868S**	PV PV	EDD 47 mm, EF 31% SD at 58	2 brothers with DCM deceased Son with EDD 56 mm and EF 45%	8	3	5	1
5	52	F	No	DSP-V1530F KCNQ1-L496Q	VUS VUS	EDD 61 mm, EF 40% CPA at 52, QTc 520 ms	Mother SD at 68	4	0	0	0
6	40	M	Yes (47)	**TTN-Y17457*** DSC2-T268A	PV VUS	EDD 68 mm, EF 33% Alcohol (excessive)	Brother with DCM, deceased	6	0	2	0

Table 2. Clinical and family characteristics of the 87 genotyped patients with dilated cardiomyopathy.

Patient	Age	Sex	Tx (age)	Genetic variant	Classification of variants	Clinical data	Familial history	Relatives assessed	Relatives affected	Carrier relatives	Carriers with phenotype
7	54	M	No	**ACTN2-A732T**	PPV	EDD 61 mm, EF 31% Alcohol (excessive), AF	Sister with PM, cardiac aunt who died young	5	0	2	0
8	74	F	No	**LDB3-S203W** (homozygous)	PPV	EDD 50 mm, non-compaction	Mother died at 34, uncle SD at 71	0	0	0	0
9	66	M	No	DSP-c.273 + 5G>A MYPN-R377Q TTN-A4529T	VUS VUS VUS	EDD 64 mm, EF 35%, non-compaction	Without interest	2	0	0	0
10	34	M	No	**MYH7-A990T** FLNC-V300M LAMA4-A64V	PPV VUS VUS	EDD 67 mm, EF 29%	Father with DCM and mild disease	3	1	1	1
11	41	F	No	**DSP-g.32449G>T** PKP2-E58D	PV VUS	EDD 54 mm, EF 30% Postpartum, NSVT	Grandma SD at 67	5	0	2	0
12	60	F	Yes (65)	DSG2-P142T TTN-R1670H	VUS VUS	EDD 71 mm, EF 16%	Sister with DCM and Tx at 61, another sister PM and low EF	5	2	5	3
13	56	M	Yes (60)	–		EDD 82 mm, EF 20% NSVT	Brother with DCM and Tx, nephew with ataxia and Tx at 9	2	2	0	0

Table 2. Clinical and family characteristics of the 87 genotyped patients with dilated cardiomyopathy.

Patient	Age	Sex	Tx (age)	Genetic variant	Classification of variants	Clinical data	Familial history	Relatives assessed	Relatives affected	Carrier relatives	Carriers with phenotype
14	23	M	Yes (26)	**MYBPC3-E1085Q** **MYBPC3-M854I**	PPV PPV	EDD 59 mm, EF 46%	Grandpa SD at 45	0	0	0	0
15	60	M	Yes (62)	**MYBPC3-D605G** RYR2-T854I KCNH2-E289Q	PP VUS VUS	EDD 79 mm, EF 19% QTc 420 ms	Father with unspecified heart disease	3	0	3	0
16	51	M	No	**LMNA-R321***	PV	EDD 65 mm, EF 42% AF	Father SD at 61 (pending PM), uncle SD at 35, cousin Tx	4	1	3	1
17	52	F	No	–		EDD 52 mm, EF 23%	Father with ischaemic DCM	0	0	0	0
18	53	M	Yes (64)	**DSG2-E399K**	PPV	EDD 60 mm, EF 22% Alcohol (mild), AF	Without interest	2	0	1	0
19	39	M	No	–		EDD 74 mm, EF 28%	Mother with short PR and PST 2 daughters with short PR	5	1	0	0
20	57	M	Yes (61)	–		EDD 68 mm, EF 17% Alcohol (mild), AF	Brother with DCM Son with DCM after myocarditis	6	2	0	0

Table 2. Clinical and family characteristics of the 87 genotyped patients with dilated cardiomyopathy.

Patient	Age	Sex	Tx (age)	Genetic variant	Classification of variants	Clinical data	Familial history	Relatives assessed	Relatives affected	Carrier relatives	Carriers with phenotype
21	34	M	No	–		EDD 56 mm, EF 35% Low voltages, NSVT	Father with DCM and Tx at 61 Uncle with DCM	6	2	0	0
22	64	M	Yes (65)	**RBM20-Q176E** TTN-N8465S	PPV VUS	EDD 97 mm, EF 27%	Without interest	0	0	0	0
23	33	M	No	–		EDD 67 mm, EF 19% Alcohol (mild), QTc 453 ms	Without interest	0	0	0	0
24	60	M	Yes (63)	–		EDD 74 mm, EF 20% Alcohol (excessive)	Several relatives with DCM, niece SD at 15	13	6	0	0
25	7	M	Yes (7)	**DSP-S1754R** KCNQ1-Q530H TTN-V4667M	PPV VUS VUS	Severely depressed EF	Sister died at 8	4	0	1	0
26	56	F	No	**MYPN-V1255M**	PPV	EDD 67 mm, EF 39%	Without interest	1	0	0	0
27	17	M	No	–		EDD 63 mm, EF 36%	Cardiac father SD at 64	1	0	0	0
28	56	M	No	TTN-T23707I	VUS	EDD 82 mm, EF 19%	Uncle with possible DCM	0	0	0	0

Table 2. Clinical and family characteristics of the 87 genotyped patients with dilated cardiomyopathy.

Patient	Age	Sex	Tx (age)	Genetic variant	Classification of variants	Clinical data	Familial history	Relatives assessed	Relatives affected	Carrier relatives	Carriers with phenotype
29	56	F	No	–		EDD 52 mm, EF 47%, non-compaction, bicuspid AoV	Without interest	6	0	0	0
30	49	M	No	–		EDD 66 mm, EF 22% Alcohol (mild)	First cousin DCM died at 19	5	1	0	0
31	58	M	No	–		EDD 56 mm, EF 27%	Mother SD at 67	0	0	0	0
32	56	M	No	**TTN-E3810*** FLNC-R421W KCNH2-D982V	PV VUS VUS	EDD 59 mm, EF 39%	Mother with DCM lives at 83 Brother with DCM and ICD	3	2	2	1
33	30	M	No	**DMD-V2305N*5**	PV	EDD 62 mm, EF 21%	Without interest	1	0	1	0
34	47	M	Yes (56)	LAMA2-C738S TMPO-T319I	VUS VUS	EDD 72 mm, EF 20%	Grandmother and father with DCM	2	2	0	0
35	3	M	No	DSG2-Q1114* TBX20-F113S	VUS VUS	EDD 55, EF lower limits	Uncle with DCM	4	1	2	0
36	25	M	No	–		EDD 67 mm, EF 16% AF	Without interest	0	0	0	0

Table 2. Clinical and family characteristics of the 87 genotyped patients with dilated cardiomyopathy.

Patient	Age	Sex	Tx (age)	Genetic variant	Classification of variants	Clinical data	Familial history	Relatives assessed	Relatives affected	Carrier relatives	Carriers with phenotype
37	19	M	Yes (19)	**TTN-P18608Qfs*3** BAG3-C151H	PV VUS	EDD 68 mm, EF 22% High CPK	Without interest	5	0	0	0
38	60	M	No	**TPM1-D230N**	PV	EDD 77 mm, EF 18% NSVT	2 siblings and a daughter with DCM	7	3	2	2
39	68	F	Yes (68)	–		EDD 93 mm, EF 20% AF	Without interest	0	0	0	0
40	46	M	Yes (50)	–		EDD 79 mm, EF 26% Alcohol (moderate)	Cardiac mother died at 65	5	0	0	0
41	56	M	No	**MYBPC3-E838Q** SCN5A-H445D	PPV VUS	EDD 65 mm, EF 18% Alcohol (former)	Mother with DCM died at 72	5	1	2	1
42	43	M	No	**TTN-W13407*** SCN5A-Q1832	PV VUS	EDD 57 mm, EF 40%	Father with DCM died at 45, affected son	3	2	2	1
43	52	M	Yes (65)	**TTN-Y9697*** **DSG2-P629S** **DSG2-G678A**	PV PPV PPV	EDD 63 mm, EF 18% AF, NSVT	Father died at 57	3	1	3	1

Table 2. Clinical and family characteristics of the 87 genotyped patients with dilated cardiomyopathy.

Patient	Age	Sex	Tx (age)	Genetic variant	Classification of variants	Clinical data	Familial history	Relatives assessed	Relatives affected	Carrier relatives	Carriers with phenotype
44	57	M	Yes (61)	**TTN-N4176***	PV	EDD 66 mm, EF 18% AF, NSVT	Without interest	6	0	1	0
45	43	F	Yes (50)	**DSP-I874M**	PPV	EDD 85 mm, EF 12%	Father passed away at 51	11	0	0	0
46	36	M	Yes (36)	TTN-D6527del SCN5A-R1898H	VUS VUS	EDD 74 mm, EF 20% QTc 452 ms	Without interest	0	0	0	0
47	44	M	Yes (61)	TTN-D14478V	VUS	EDD 64 mm, EF 20% AF	Without interest	5	0	0	0
48	49	F	No	**LMNA-R377H**	PV	EDD 51 mm, EF 46%	Father died at 40 (AMI?)	7	0	1	0
49	40	M	No	**TTN-V16403Efs*33**	PV	EDD 63 mm, EF 21%	Brother SD at 36	2	0	1	0
50	60	M	No	**TTN-R4375***	PV	EDD 62 mm, EF 27%, non-compaction	Without interest	2	0	1	0
51	41	M	Yes (42)	**TTN-R8272***	PV	EDD 68 mm, EF 25%	Daughter with DCM at 16	2	1	2	1
52	60	F	No	RYR2-R3084Q TTN-V19064F	VUS VUS	EDD 53 mm, EF 35%	Sister with DCM	3	1	0	0

Table 2. Clinical and family characteristics of the 87 genotyped patients with dilated cardiomyopathy.

Patient	Age	Sex	Tx (age)	Genetic variant	Classification of variants	Clinical data	Familial history	Relatives assessed	Relatives affected	Carrier relatives	Carriers with phenotype
53	33	M	No	TTN-S1330L	VUS	EDD 60 mm, EF 49% Alcohol (mild)	Without interest	1	0	0	0
54	51	F	No	**TTN-T300Dfs*23**	PV	EDD 62 mm, EF 24%	Brother with EF 40% and bicuspid AoV	3	0	0	0
55	42	M	No	**DES-R275G**	PPV	EDD 57 mm, EF 45% CPA at 42	Son with hypertrabeculation already normalised	1	0	0	0
56	68	F	No	–		EDD 65 mm, EF 21%, non-compaction, QTc 457 ms	Without interest	1	0	0	0
57	44	M	Yes (44)	PKP2-R811S	VUS	EDD 71 mm, EF 32% Kidney Tx, alcohol (moderate)	Brother with HCM	2	0	0	0
58	59	M	No	LAMA2-Q1174* TTN-R19374Q	VUS VUS	EDD 71 mm, EF 29% NSVT	2 siblings with DCM (one SD at 57), nephew Tx at 28	8	3	0	0
59	48	M	No	MYBPC3-A216T FLNC-R1657L	VUS VUS	EDD 61 mm, EF 26%	Son with mild hypertrabeculation	4	0	0	0
60	41	M	No	**MYH7-R1697W**	PPV	No data	Without interest	3	0	0	0

Table 2. Clinical and family characteristics of the 87 genotyped patients with dilated cardiomyopathy.

Patient	Age	Sex	Tx (age)	Genetic variant	Classification of variants	Clinical data	Familial history	Relatives assessed	Relatives affected	Carrier relatives	Carriers with phenotype
61	20	F	No	**DSP-D1106***	PV	EDD 53 mm, EF 31% Low voltages, NSVT. WPW	Grandmother DCM and SD at 61	3	1	0	0
62	44	M	Yes (52)	TNNT2-c.42-6T>C DSP-L2103E	VUS VUS	EDD 74 mm, EF 22% Alcohol (moderate), NSVT	Brother DCM waiting for Tx	3	1	0	0
63	50	M	Yes (54)	**LMNA-M1L**	PPV	EDD 53 mm, EF 20%	Mother with DCM	6	1	3	1
64	49	M	Yes (49)	TTN-D14478V	VUS	EDD 82 mm, EF 23%	Father with DCM died at 45	7	1	0	0
65	64	F	No	–		EDD 65 mm, EF 26% Mild AS	Sister with DCM and Tx	6	1	0	0
66	47	M	Yes (50)	RYR2-N198I	VUS	EDD 58 mm, EF 28% PM at 45, NSVT	Brother with HCM and PM, mother SD at 63	8	0	0	0
67	26	M	Yes (28)	**RBM20-R711C**	PPV	EDD 84 mm, EF 22% Alcohol (moderate)	Mother with DCM	0	0	0	0

Table 2. Clinical and family characteristics of the 87 genotyped patients with dilated cardiomyopathy.

Patient	Age	Sex	Tx (age)	Genetic variant	Classification of variants	Clinical data	Familial history	Relatives assessed	Relatives affected	Carrier relatives	Carriers with phenotype
68	34	F	Yes (35)	**TTN-R17841*** DSP-T760R	PV VUS	EDD 68 mm, EF 33%	Father with DCM and SD at 55	3	0	0	0
69	32	M	Yes (35)	**TNNI3-I132T** LMNA-G523R MYBPC3-P873L	PPV VUS VUS	EDD 60 mm, EF 14%	Without interest	0	0	0	0
70	1	F	No	**FLNC-Pro963Rfs*26 DSG2-C813R**	PV PPV	EDD 49 mm, EF 20%	Mother with mild dilatation and systolic dysfunction	4	0	2	0
71	1	M	No	**MYH7-E344K**	PPV	EDD 49 mm, EF 36%	First cousin with postnatal myocarditis	2	0	1	0
72	59	M	No	SCN5A-P1090L	VUS	EDD 61 mm, EF 38%, non-compaction, NSVT	Son with AMI at 36	0	0	0	0
73	54	M	Yes (58)	**MYBPC3-K505del**	PV	EDD 62 mm, EF 30% Died during Tx	Without interest	3	0	3	0
74	40	F	No	TTN-R12768T	VUS	EDD 54 mm, EF 48% AF	Brother with SD at 39, son with mild hypertrabeculation	8	0	0	0
75	14	M	Yes (14)	–		EDD 70 mm, EF 12%	Father with septal hypertrophy	3	0	0	0

Table 2. Clinical and family characteristics of the 87 genotyped patients with dilated cardiomyopathy.

Patient	Age	Sex	Tx (age)	Genetic variant	Classification of variants	Clinical data	Familial history	Relatives assessed	Relatives affected	Carrier relatives	Carriers with phenotype
76	40	F	Yes (40)	–		No data	Aunt with SD at 56	2	0	0	0
77	34	M	No	**MYH7-V338M**	PV	Restrictive phenotype, AF	Father SD at 29	5	0	2	0
78	56	F	No	RYR2-V4634A	VUS	EDD 66 mm, EF 33%	Cardiac brother died at 29	0	0	0	0
79	46	F	No	PKP2-c.1510° + 5G> A	PV	EDD 59 mm, EF 28% Alcohol (mild)	Father with DCM and SD at 67, 2 uncles with DCM	4	0	0	0
80	34	M	No	MYH7-I724T	PPV	EDD 76 mm, EF 25%, NSVT SD at 44	Aunt died at 40, daughter with EF 48%	3	1	1	1
81	46	M	No	TTN-R8272Q	VUS	EDD 74 mm, EF 26%	Mother with LBBB	4	0	0	0
82	39	M	Yes (46)	NEXN-E470del TTN-P8106S	VUS VUS	EDD 72 mm, EF 28% Alcohol (moderate)	Brother died at the age of one month, son with LVH	0	0	0	0
83	24	M	Yes (28)	DSP-R1184Q	VUS	EDD 65 mm, EF 24% NSVT	Father with moderate septal hypertrophy	0	0	0	0

Table 2. Clinical and family characteristics of the 87 genotyped patients with dilated cardiomyopathy.

Patient	Age	Sex	Tx (age)	Genetic variant	Classification of variants	Clinical data	Familial history	Relatives assessed	Relatives affected	Carrier relatives	Carriers with phenotype
84	41	M	Yes (42)	–		EDD 73 mm, EF 24% PM	Grandfather died at 54	2	0	0	0
85	40	M	Yes (40)	TTN-A20786D	VUS	EDD 53 mm, EF 46%, possible ischaemic heart disease	Without interest	1	0	0	0
86	50	M	No	MYH7-K910R	PPV	EDD 62 mm, EF 45%	Brother and several cousins with DCM	10	4	3	1
87	41	M	Yes (43)	TTN-V17206I TTN-V9377I	VUS VUS	EDD 81 mm, EF 33% Alcohol (moderate)	Brother with cardiomegaly	0	0	0	0

LBBB: left bundle branch block; CPK: creatine phosphokinase; LVH: left ventricular hypertrophy; ICD: implantable cardioverter defibrillator; EDD: end-diastolic diameter; AF: atrial fibrillation; EF: ejection fraction; AMI: acute myocardial infarction; F: female; DCM: dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; PM: pacemaker; SD: sudden death; CPA: cardiopulmonary arrest; PST: paroxysmal supraventricular tachycardia; NSVT: nonsustained ventricular tachycardia; Tx: transplantation; M: male; PV: pathogenic variant; PPV: potentially pathogenic variant; VUS: variant of uncertain significance.

Table 3. Identified genetic variants classified as pathogenic.

Gene	Patient	Mutation	Type	Described	GnomADMAF (%)	Comments on the classification of their pathogenicity
DMD	33	p.Val2305Asnfs*5/g.1416999_1507494 delinsAATAG	Frameshift	No	–	A truncating variant, not present in controls. Hotspot region for deletions in DMD
DSP	11	c.2131-1G>T/g.32449G>T	Splicing	No	–	Intronic variant, not previously described. Bioinformatic predictors support pathogenicity, not in controls
DSP	61	Glu1106*/g.37870G>T	Nonsense	No	–	A truncating variant, not present in controls. Core domain of the protein (rod domain)
FLNC	70	p.Pro963Argfs*26/g.13444delC	Frameshift	Yes	–	A truncating variant, not present in controls. Previously described by our group23
LMNA	16	p.Arg321*/g.21219C>T	Nonsense	Yes	<0.001	Described in medical literature, proven cosegregation. Considered pathogenic in ClinVar, very low frequency in controls
LMNA	48	p.Arg377His/g.21388G>A	Missense	Yes	–	Described in medical literature, proven cosegregation. Functional studies and bioinformatics predictors support pathogenicity, not in controls
MYBPC3	73	p.Lys505del/g.11011_11013delAAG	Inframe	Yes	<0.001	Described in medical literature, proven cosegregation. Functional studies and bioinformatics predictors support pathogenicity, very rare in controls
MYBPC3	4	p.Gly868Ser/g.16312G >A	Splicing	Yes	0.0037	Intronic variant, described in the medical literature. Bioinformatic predictors and functional studies support pathogenicity, low frequency in controls
MYH7	77	p.Val338Met/g.6387G>A	Missense	Yes	–	Described in medical literature, proven cosegregation. Bioinformatic predictors support pathogenicity. Motor domain, not in controls

Table 3. Identified genetic variants classified as pathogenic.

Gene	Patient	Mutation	Type	Described	GnomADMAF (%)	Comments on the classification of their pathogenicity
TTN	54	p.Thr300Aspfs*23/g.7919_7920insT	Frameshift	No	–	A truncating variant, not present in controls. Exon 6, located in Z-disc, affects all isoforms of the protein
TTN	32	p.Glu3810*/g.66708G>T	Frameshift	No	–	A truncating variant, not present in controls. Exon 49, located in band i, affects all isoforms except N2A and Novex-3
TTN	44	p.Asn4176*/g.67801_67802insT	Nonsense	No	–	A truncating variant, not present in controls. Exon 49, located in band i, affects all isoforms except N2A and Novex-3
TTN	50	p.Arg4375*/g.69183C>T	Nonsense	No	–	A truncating variant, not present in controls. Exon 50, located in band i, affects all isoforms except Novex-3
TTN	51	p.Arg8272*/g.198123C> T	Nonsense	No	–	A truncating variant, not present in controls. Exon 274, located in band A, affects all isoforms except Novex-3
TTN	43	p.Tyr9697*/g.207809T>G	Nonsense	No	–	A truncating variant, not present in controls. Exon 290, located in band A, affects all isoforms except Novex-3
TTN	42	p.Trp13407*/g.227642G>A	Nonsense	No	–	A truncating variant, not present in controls. Exon 320, located in band A, affects all isoforms except Novex-3
TTN	49	p.Val16403Glufs*33/g.237695delT	Frameshift	No	–	A truncating variant, not present in controls. Exon 327, located in band A, affects all isoforms except Novex-3
TTN	4	p.Lys16797Argfs*25/g.238876delA	Frameshift	No	–	A truncating variant, not present in controls. Exon 327, located in band A, affects all isoforms except Novex-3
TTN	6	p.Tyr17457*/g.240858T>G	Nonsense	No	<0.001	A truncating variant, very low frequency in controls. Exon 327, located in band A, affects all isoforms except Novex-3
TTN	68	p.Arg17841*/g.242008C>T	Nonsense	Yes	<0.001	A truncating variant, very low frequency in controls. Exon 327, located in band A, affects all isoforms except Novex-3

Table 3. Identified genetic variants classified as pathogenic.

Gene	Patient	Mutation	Type	Described	GnomADMAF (%)	Comments on the classification of their pathogenicity
TTN	37	p.Pro18608Glnfs*3/g.244308_ 244309delACinsT	Frameshift	No	–	A truncating variant, not present in controls. Exon 327, located in band A, affects all isoforms except Novex-3
TPM1	38	p.Asp230Asn/g.19625G>A	Missense	Yes	–	Described in medical literature, not in controls. Proven cosegregation, positive functional studies. Discordant bioinformatics predictors
PKP2	79	c.1510 + 5G>A/g.53670G>A	Splicing	No	–	Intronic variant, not previously described. Bioinformatic predictors support pathogenicity, not in controls

Table 4. Identified genetic variants classified as possibly pathogenic.

Gene	Patient	Mutation	Type	Described	GnomADAF (%)	Comments on the classification of their pathogenicity
ACTN2	7	p.Ala732Thr/g.71027G>A	Missense	Yes	0.0041	Missense variant, described in the medical literature, low frequency in controls. Bioinformatic predictors support pathogenicity
DES	55	p.Arg275Gly/g.2206A>G	Missense	No	–	Missense variant, not present in controls, bioinformatics predictors support pathogenicity. It is located in helix 2A of the central rod domain
DSP	45	p.Ile874Met/g.33844C>G	Missense	No	0.0041	Missense variant, low frequency in controls, bioinformatics predictors support pathogenicity. Globular N-terminal region of the protein
DSP	3	p.Arg925Pro/g.34801G>C	Missense	No	–	Missense variant, not present in controls, discordant bioinformatics predictors. Located in the globular N-terminal region of the protein
DSP	25	p.Ser1754Arg/g.39816C>G	Missense	No	–	Missense variant, not present in controls, negative bioinformatics predictors. Located in the core domain of the protein (rod domain)
DSG2	18	p.Glu399Lys/g.32959G>A	Missense	No	0.0033	Missense variant, low frequency in controls. Bioinformatic predictors support pathogenicity. Cadherin 4 domain of the protein
DSG2	43	p.Pro629Ser/g.42990C>T	Missense	No	0.0017	Missense variant, low frequency in controls. Bioinformatic predictors support pathogenicity. Transmembrane region of the helical structure
DSG2	43	p.Gly678Ala/g.44343G>C	Missense	Yes	0.0041	Missense variant described in the medical literature, low frequency in controls. Negative bioinformatics predictors. C-terminal cytoplasmic domain
DSG2	70	Cys813Arg/g.47615T>C	Missense	Yes	–	Missense variant described in the medical literature, not in controls. Bioinformatic predictors support pathogenicity. Intracellular cadherin segment
LDB3	8	p.Ser203Trp/g.13159C>G	Missense	No	–	Missense variant, not present in controls. Bioinformatic predictors support pathogenicity. ZASP-like domain
LMNA	63	Met1Leu/g.213A>T	Missense	No	–	Missense variant, not present in controls. A bioinformatics study predicts that it would affect the signal peptide, a mechanism already described in other variants

Table 4. Identified genetic variants classified as possibly pathogenic.

Gene	Patient	Mutation	Type	Described	GnomADAF (%)	Comments on the classification of their pathogenicity
LMNA	2	p.Glu124Lys/g.15924G>A	Missense	No	–	Missense variant, not in controls. Computational predictors support pathogenicity. MLIP interaction region
MYBPC3	15	p.Asp605Gly/g.12482a>G	Missense	Yes	0.0146	Missense variant described in the medical literature, low population frequency. Discordant bioinformatics predictors, protein C4 domain
MYBPC3	41	p.Glu838Gln/g.16222G>C	Missense	Yes	<0.001	Missense variant described in the medical literature, very low population frequency. Positive bioinformatics predictors. Protein C6 domain
MYBPC3	14	p.Met854Ile/g.16272G>A	Missense	No	<0.001	Missense variant, very low frequency in controls. Discordant bioinformatics predictors. Protein C6 domain
MYBPC3	14	p.Glu1085Gln/g.20432G>C	Missense	Yes	–	Missense variant, described in the medical literature. Not in controls. Bioinformatic predictors support pathogenicity. Protein C9 domain
MYH7	2	p.Ser1102Thr/g.15299G>C	Missense	No	–	Missense variant, not present in controls. Negative bioinformatics predictors. S2 subfragment of protein
MYH7	71	p.Glu344Lys/g.6405G> A	Missense	No	–	Missense variant, not in controls. Discordant bioinformatics predictors. Core subdomain of protein
MYH7	80	p.Ile724Thr/g.10478T>C	Missense	No	–	Missense variant, not in controls. Positive bioinformatics predictors. Protein converting region, functionally highly relevant
MYH7	86	p.Lys910Arg/g.12188a>G	Missense	No	–	Missense variant, not in controls. Discordant bioinformatics predictors. MYBPC3 binding region, in the tail of the protein
MYH7	10	p.Ala990Thr/g.12610G>A	Missense	No	0.0008	Missense variant, low frequency in controls. Discordant bioinformatics predictors. Fragment S2, in the tail of the protein
MYH7	60	p.Arg1697Trp/g.20591C>T	Missense	No	<0.001	Missense variant, very low frequency in controls. Positive bioinformatics predictors. Protein tail, “f” position

Table 4. Identified genetic variants classified as possibly pathogenic.

Gene	Patient	Mutation	Type	Described	GnomADAF (%)	Comments on the classification of their pathogenicity
MYPN	26	p.Val1255Met/g.97381G>A	Missense	No	0.0041	Missense variant, low frequency in controls. Positive bioinformatics predictors. Fifth immunoglobulin-like domain of the C-terminal region
RBM20	22	p.Gln176Glu/g.141739C>G	Missense	No	–	Missense variant, not in controls. Discordant bioinformatics predictors. Variant described in the medical literature in contiguous amino acid
RBM20	67	p.Arg711Cys/g.173132C>T	Missense	No	<0.001	Missense variant, very low frequency in controls. Positive bioinformatics predictors. Located in hotspot region
TNNI3	69	p.Ile132Thr/g.4549T>C	Missense	No	–	Missense variant, not in controls. Discordant bioinformatics predictors. Protein inhibitory región