Clinical Profile of Cardiac Involvement in Danon Disease: A Multicenter European Registry.

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Abstract

Aims

Danon disease is a rare X-linked dominant metabolic disorder leading to cardiomyopathy, proximal myopathy and intellectual disability. To date, no specific therapy is available to prevent disease progression. The purpose of this registry is to generate a comprehensive picture of the prevalence, clinical presentation and outcome of patients with Danon disease evaluated in the cardiomyopathy centers over Europe.

Methods and Results

Data was retrospectively collected in cardiology centers over Europe using a consensus datasheet. Clinical and genetic data were analyzed. The cohort comprised 65 patients; 46% (N = 30) were males and 54% (N = 35) were females, including 27 affected and 8 gene carriers. The age at diagnosis was birth to 42 years in males and 2 to 65 years in females ($p<0.01$). Mean age at first heart failure hospitalization was lower 18 ± 6 in males vs. 28 ±17 in females, $p<0.003$. Cardiac involvement was seen in 55 of our patients (96%). Left ventricular (LV) hypertrophy was reported in 73% of males and in 74% of the affected females with an average left ventricular wall thickness of 20±8 and 17±6 mm respectively. Reduced LV function was reported in 30% males and 59% of females with mean LVEF of 34±11% and 28±13% respectively. The principal cause of death was heart failure (n=XX). Systolic LV dysfunction was the main predictor of adverse outcome. Sixteen (28%) of the patients underwent heart transplantation or received a left ventricular assist device (LVAD). There was a 62% (5/8) mortality in males receiving heart transplant or LVAD within 1 year after the procedure.

Conclusion

Comentado [PGP1]: This is mean LVEF of the entire cohort or just of the patients with reduced LVEF

Comentado [PGP2]: In results it says 16 Htx/LVADs

DD presents approximately 10-20 years earlier in males than females and runs a highly malignant course in both genders. Cardiac manifestations are comparable among genders with high individual variability. Extra-cardiac manifestations are uncommon in females, making their clinical diagnosis unlikely.

Keywords: Danon Disease, cardiomyopathy , arrhythmia, heart failure, heart transplantation

Introduction

Danon disease is a rare X-linked dominant metabolic disorder described first by Danon et al in 1981 in two unrelated 16-year-old boys with mental retardation, cardiomegaly, proximal myopathy and intellectual disability (1). In 1940, Antopol et al (2) reported two brothers with cardiac hypertrophy caused by glycogen storage in cardiac and skeletal muscles, leading to death in the second decade of life. This is apparently the earliest description of this rare and lethal condition. Danon's, also termed *Glycogen Storage Disease IIb*, is caused by *LAMP2* mutations is a. Given the role of LAMP2 in lysosomal fusion (3), LAMP2 deficiency leads to failure to complete the final step of the autophagic process, where digestion of aged cellular contents (inc. glycogen) and organelles takes place. Inability to remove aged mitochondria (mitophagy) leads to mitochondrial dysfunction, energetic deficiency and oxidative stress (4–6). Although Danon is a multisystem disease, cardiac manifestations constitute the main cause of morbidity and mortality. In recent years, the disease has been diagnosed with higher frequency due to the introduction of modern genetic techniques. To date, no specific therapy is available to prevent disease progression.

Comentado [PGP3]: ???

International registries are crucial in studying orphan diseases to properly define the natural history, outcome of interventions and potentially enroll patients in therapeutic trials. The purpose of this registry is to generate a comprehensive picture of the prevalence, clinical presentation and outcome of patients with Danon disease evaluated in the cardiomyopathy centers over Europe, Our data should help improve the recognition and diagnosis of this complex disease, allowing to optimize the medical care of patients and families.

Methods

The study is a collaborative effort of a team of expert cardiologists across Europe who operate within a framework specialized centers dedicated to cardiomyopathies and inherited

heart diseases. Data was collected retrospectively using a consensus datasheet and interpreted by the primary investigators. All patients had to be evaluated and followed by a cardiologist in the participating center. Patient management and data collection were conducted according to local regulations.

The diagnosis of Danon disease was based of clinical manifestations and documentation of a mutation in the LAMP2 gene. Carrier status (observed only in females) was defined by confirmation of a disease-causing mutation in the absence of any (cardiac or extracardiac) evidence of disease, and the presence of one or more affected first-degree family members. Patients and carriers were classified according to gender, age of diagnosis/presentation, clinical features at the time of first evaluation and cardiovascular events during follow-up. Mutations were classified according to their principal type: mutations affecting a Splice-site, Frameshift, Non-sense mutations, or a Major deletion. Patient follow-up and management were conducted according to the local medical practice. The data on genetic and clinical profile included in the database were collected by the assigned cardiologist. The presented data includes clinical, electrocardiographic and echocardiographic features at initial evaluation (as reported by the participating centers) as well as hard outcome events such as cardiac arrest, device implantation, heart transplantation and death.

Statistical analysis

Variables were described according to their properties. Categorical variables are reported in frequencies and percentages, and significance was assessed using the chi-square test or Fischer's exact test. Continuous variables with a normal distribution were reported as mean and standard deviation values, and significance was assessed using the t-test. Continuous variables that did not have a normal distribution were reported as median and interquartile range (IQR, 25th-75th percentiles) values, and significance was assessed using non-

parametric Mann-Whitney U test. All statistical tests were 2-sided, and a p value of less than 0.05 was considered significant. In the adjusted analysis, potential covariates were analyzed in univariate logistic regression models, and variables found to have a $p < 0.1$ were included in the adjusted model. The statistical analysis was carried out with the use of R version 3.6.1 software (The R Foundation) and R-studio 1.2.5001 (R Studio, Inc.).

A comprehensive bibliographical search (PubMed, Web of Science and Google Scholar) collected all available clinical information of families and individuals who carried LAMP2 mutations. Curves illustrating the cumulative percentage of clinically affected carriers of pathogenic variants in LAMP2 (i.e. Danon disease) against age as well as Kaplan-Meier survival curve were constructed to correspond to patients from this project, and to all patients in Health in Code's database.

Results

Epidemiology and Genetics

Of 65 patients included, 46% (N = 30) were males and 54% (N = 35) were females including 27 affected and 8 gene carriers (Table 1). Twenty nine families from 8 countries over Europe, harboring 25 different mutations in the LAMP2 gene, including 9 frameshift, 8 nonsense, 7 affecting a splice-site, and one major deletion. Among the clinically affected patients, the most common variants were mutations affecting a splice site (Suppl, Table 1). Mutations appeared to arise de novo in 13 individuals. Family history of sudden death was reported in 13/29 (45%) representing a majority among those with *familial* disease. There was no significant difference when comparing nonsense to frameshift and splice-site mutations regarding age of diagnosis and age of death or heart transplant no was found.

There was a significant difference in the age at diagnosis: mean 13 years in males (ranging from birth to 42), compared to 36 years in the affected females (range2-65 yr., $p<0.01$). The mean age of female carriers was 31 years (range 2-46). The age of first heart failure hospitalization was also lower in males compare to females (18 ± 6 vs 28 ± 16.6, $p<0.003$, Figure 1).

Clinical Manifestations by Gender

Cardiac involvement was seen in 55 of our patients (96%). Cardiomyopathy was present in all the affected except two of the males (Table 2). Left ventricular hypertrophy (wall thickness ≥13 mm) was reported in 73 % (n=22) of males and in 74% (n=20) of females with an average left ventricular wall thickness of 20±8 and 17±6 (mm) respectively. Hypokinetic (EF <50%) cardiomyopathy (dilated or hypertrophic) presented in 30% (n=12) of males and 59% (n=16) of females with mean ejection fraction of 34±11% and 28±13% respectively.

Electrophysiological abnormalities such as extreme ECG voltage, Wolf Parkinson White (WPW) and early atria-ventricular block are considered Red Flags suggesting a possibility of metabolic disease being the cause of cardiomyopathy (7). In our cohort, conduction abnormalities such as WPW pattern were quite common in males (50%,n=15) and females (41%, n=11, Table 2). Atria-ventricular block was most commonly of the 1st degree. Advanced atria-ventricular block was reported in 15% (n=4) in males and 14% (n=4) of the affected females. Paroxysmal or permanent atrial fibrillation was more common in females (55% vs 27% in males, p=0.02). Lethal ventricular tachyarrhythmia's (sustained ventricular tachycardia, ventricular fibrillation or cardiac arrest) were reported in males and females. A total of 39 (68%) patients received device therapy (permanent pacemaker, ICD, or CRT-D, Table 2). The most common indication for ICD implantation in the normal EF group was primary prevention. Two patients received ICD after aborted ventricular fibrillation and 2

ICD after a documented episode of sustained ventricular tachycardia. Among patients within the reduced EF group, 7 had ventricular tachycardia early after the diagnosis of Danon disease while 4 reported a recorded lethal ventricular arrhythmia and appropriate shock therapy during the follow-up.

Other Clinical Features

Extracardiac manifestation seen in Danon disease aggravate the morbidity and may constitute a clue to the diagnosis. Muscular involvement in a form of myopathy was clinically significant in 60% (n=18) of males but in only 3% (n=1) in females ($p<0.001$). Elevated creatine phosphokinase (>2 times normal limit) was reported in 80% (n=24) of males and none of the women ($p<0.001$). Elevated liver transaminases enzymes were found in 83% (n=25) of males and 6% (n=2) in females ($p<0.001$). Pronounced learning, neurological or psychiatric problems were quite common in 73% (n=22) in males but rare in 9% (n=3) of females ($p<0.001$). Clinically manifest visual involvement was equally uncommon in both gender group (Figure 2).

Mortality and Prognosis

In our cohort, among 57 clinically affected, 16 patients (27%) underwent heart transplantation or received a left ventricular assist device (LVAD). Eight were males (6 underwent heart transplantation and 2 had LVAD implanted, mean age 23±8 yr.) and eight were females (all underwent heart transplantation at mean age 29±9 yr, $p=0.13$, Figure 1).

Seventeen patients (30%, 11 males and 6 females) died over a total follow up of 350 patient-years, including 5 heart transplant/assist device male patients. The mean age of death among men were 23±19 and 45±18 in females ($p=0.02$). The leading cause of death was heart failure, that was seen in 10 patients (59%). Sudden cardiac death occurred in 2 female

patients who had no ICD. Four died from malignant ventricular arrhythmias despite having an implanted cardioverter-defibrillator (ICD).

Five male heart transplant (n=4) /LVAD recipients (n=1) died, all within 1 year after the procedure, comprising a 62% mortality. No death was reported among females receiving heart transplant.

Comentado [PGP4]: Causes of death?

Overall prognosis was independent of gender. In a univariate analysis, patients who had a terminal event (death, assist device or heart transplantation) were older at diagnosis and at the time of evaluation (Table 3 and suppl. Table 2). Reduced left ventricular systolic function has associated with mortality (71% vs. 31%, p=0.002), as were AV block >1st degree (25% vs. 3%, p=0.025) and atrial fibrillation (56% v. 28%, p=0.034).

In an adjusted logistic model, which includs variables that were significant in a univariate analysis, only hypokinetic left ventricle remained a significant predictor of mortality. This result did not change when we included also age and gender in the multivariate model (OR 3.85, 95%CI 1.06-14.96, p=0.043, Table 3).

Disease Expression and Survival Curves

We then constructed the curves of cumulative incidence of disease expression and survival by age. We then compared our cohort to all DD cases reported in the literature (Figures 3a and 3b comprising data from 105 males and 90 females). There is no notable difference between the curves indicating that the data provided in the current cohort is highly representative of DD population in either males or females.

Discussion

Danon Disease is a rare multisystem lysosomal storage disease that is transmitted as X-Linked with dominant inheritance trait. It is caused by a dysfunction or deficiency of the

lysosome-associated membrane protein-2 (LAMP-2) that disrupts autophagy, leading to an impaired fusion of lysosomes to autophagosomes and biogenesis of lysosomes. Due to X linked dominant inheritance pattern, hemizygous males transmit the trait to all daughters but not to their sons. Heterozygous females usually suffer from milder and variable symptoms due to different patterns of X chromosome inactivation. Transmission of disease from mother to daughter is compatible with an X-linked dominant inheritance and is pronounced when the protein product of the wild-type allele remains below the normal threshold level (8). Danon disease typically occurs through LAMP2 protein deficiency but protein malfunction may explain some cases. It was actually proposed to measure LAMP2 in lymphocytes as a screening tool for DD. Different gene defects may have various effects on splicing and protein expression, but we did not find major differences in disease presentation among the mutation types (Suppl. table 1).

The real prevalence of DD in the population is unknown. Widespread screening in Genetic centers using multiple parallel /next generation sequencing (NGS) led to successful detection of LAMP-2 mutations among cardiomyopathy and myopathy patients implying a unique (often unexpected) diagnosis with grave medical and therapeutically consequences (9,10). Certain reports using NGS in highly selected cohorts from specialized referral centers has showed disease frequency of 4-6% among children with hypertrophic cardiomyopathy (HCM) (11,12), and 0.7- 4% among adults with HCM (9,13,14). The prevalence may be even higher such as 6-8% among adults with concentric HCM (15), 17-30% among patients with HCM and pre-excitation on ECG (13,16,17). It was reported to be as high as 33% among patients with vacuolar myopathy and HCM (18), suggesting that in pediatric cardiology DD is one of the leading causes of such clinical constellation (19,20). Wide application of gene sequencing also allowed identifying DD among women with cardiomyopathy, including those with early conduction system disease and a postpartum presentation. In contrast, no

Danon disease patient was identified by NGS screening among 72 children with idiopathic dilated cardiomyopathy (11).

The diagnosis of Danon is very uncommon even in cardiomyopathy clinics and this entity may not be familiar to many general cardiologists. Among 17704 studies of NGS on index cases by Health in Code laboratory (A'Coruna Spain, Lorenzo Monserrat personal communication) that included more than 10000 probands with cardiomyopathy, the evaluation of LAMP2 identified 18 pathogenic or likely-pathogenic variants.

Due to the low prevalence of the disease, most data available to date is based on small case reports and single center studies that may not represent the possible heterogeneity between families and ethnicities across the globe. Others investigators constructed datasets by collecting case series or web-based registries. Recently, Cenacchi et al.(20) reviewed all published cases of DD to date, included 332 patients reported from case reports and small series as well as 186 patients from large series. Single center studies, such as Sugie et al. (21,22) reported a total of 77 Japanese patients. Boucek et al.(23) reported 82 patients widely dispersed across four continents, of whom only 44 had clinical finding reported. López-Sainz et al. recently reported on 27 patients collected from all over Spain.(ref)

Our study represents an attempt to establish a European registry of Danon disease conducted by leading cardiomyopathy clinics across Europe. Due to disease complexity and the variable clinical course, follow up data on these patients is often incomplete. We collected data from cardiomyopathy experts who examined these patients, by using a simple questionnaire designed to target the principal points while attempting to minimize missing data. Our data on 57 genetically and clinically affected patients from Europe demonstrate a high prevalence of cardiac disease both in men (93%) and in women (100%). While there were certain

Comentado [PGP5]: Although a majority were females, we collected both male and female patients

Comentado [PGP6]: Clinical Findings and Prognosis of Danon Disease. An Analysis of the Spanish Multicenter Danon Registry.
López-Sainz Á, Salazar-Mendiguchía J, García-Álvarez A, Campuzano Larrea O, López-Garrido MÁ, García-Guereta L, Fuentes Cañamero ME, Climent Payá V, Peña-Peña ML, Zorio-Grima E, Jordá-Burgos P, Díez-López C, Brugada R, García-Pinilla JM, García-Pavía P.
Rev Esp Cardiol (Engl Ed). 2019 Jun;72(6):479-486.

differences among the genders, the main difference was in the age of presentation, but less so in the specific features of the cardiac phenotype or disease complications, While the approximate delay in disease expression in women was by approximately 20 years compared with men, the age and mode of presentation in females was highly variable

A comparable proportion of females and males presented with LVH and/or systolic dysfunction. Further, the wall thickness and the ejection fraction were similar among the genders. This finding contradicts previous reports suggesting that females mostly present with DCM compared to massive HCM in males (24). Females had a similar prevalence of ventricular arrhythmia but more atrial fibrillation, similar to smaller series reported previously (25). Sudden cardiac death among 2 female patients with no ICD is an alarming notion of their arrhythmic risk which may not necessarily be predicted by massive hypertrophy or reduced ejection fraction.

Extracardiac manifestations were common in males but rather non abundant in females, making the clinical diagnosis in affected women (in the absence of family history) nearly impossible. In depth investigations of visual and psycho-cognitive testing may disclose subtle abnormalities in the affected females but those are rarely clinically pronounced to draw attention in the early stages of the disease. Previous study reported that IQ and cognitive abilities were assessed by standardized cognitive measurements, most of them are not shown to have intellectual disability/mental retardation but rather milder cognitive (eg, executive functioning) deficits (11,26). These findings emphasize the importance of gene testing in women with what appears to be idiopathic cardiomyopathy.

Reduced left ventricular systolic function was the only independent predictor of prognosis in our study. This finding corresponds to the prevailing notion that the hallmark of cardiac Danon disease is a progressive myocardial fibrosis commencing in combined systolic and

diastolic failure as well as a substrate for ventricular arrhythmia. While novel therapies are been developed, contemporary therapies to attenuate fibrosis might be useful to modify the course of cardiomyopathy.

Our results draws attention to a poor prognosis of heart transplant in males with DD. While often no other option is left, the transplant clinic should be prepared to deal with neuromuscular complications (eg. post-transplant myopathy) and psychiatric issues which may occur in the peri- and postoperative period. While there are numerous case reports of successful heart transplantation in DD males, unlucky cases are less likely to be reported. Prospective cohorts are therefore needed to address this issue

Comentado [PGP7]: Causes of death would be very important if a case is made on this

Our data should serve as a basis to better understand the spectrum of clinical presentation and natural history of the disease and to facilitate diagnosis. Timely clinical and genetic diagnosis allow to plan observational and interventional research projects focused on treating this complex disease (30).

Limitations and perspectives

This is an observational cohort with a limited follow up. Given its retrospective nature, we had a difficulty to define the rate of disease progression as well as arrhythmia events over time. While DD heart is characterized by a progressive fibrosis we did not have an MRI data to assess its relationship to the clinical events. Our findings and conclusions should stimulate prospective observational studies to explore the critical turning points along the course of the disease(27,28) Danon disease is extremely rare and therefore at disadvantage in a stride for specific therapy. Nevertheless, attempts to develop gene therapy or (possibly) protein therapy are underway(29). Meanwhile lifestyle intervention and therapies and should be assessed in a longitudinal fashion to optimize data collection and disease management (30).

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Appendix: Cooperating Investigators

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Acknowledgements

Legends

Table 1: Title- Geographic distribution of patients and families

The distribution of Danon disease patients by countries involved in our registry, number of families and patients with gender differentiation. Last column refer to number of families detected from each country with a history of sudden cardiac death.

SCD, sudden cardiac death

Table 2: Title- Cardiac Manifestations among the affected patients

Cardiac manifestations and device implanted among the affected Danon patients are presented by gender.

LVH, Left ventricular hypertrophy (defined by , WPW,Wolf Parkinson white, VT, Ventricular tachycardia, VF, Ventricular fibrillation, ICD, Implantable cardioverter-defibrillator, CRTD, cardiac resynchronization therapy with defibrillator.

†LVH, LV wall thickness (≥13 mm), * Hypokinetic cardiomyopathy, LVEF<50%, # 2 patient expired prior to LVEF assessment

Table 3: Title- Regression analysis to characterize patients with a terminal event

The table presents the Results of logistic regression analysis. Potential variables were analyzed in univariate logistic regression models. Variables found to be significant at $p < 0.05$, were included in the multivariate model adjusted for age of diagnosis and gender.

Terminal event; Death, implantation of an assist device or heart transplant during the

follow-up

Figure 1: Title: Age at first heart failure hospitalization, heart transplantation or death

Figure shows mean age of Danon patients disease, by gender, presented with their first heart failure hospitalization, age of death and age of heart transplant or assist device implantation. Males were presented earlier with their first heart failure hospitalization compared to females (18±6 vs 28±16.6, p=0.003). Mean age of heart transplantation or assist device as well as age of death was lower in males (23±8 vs. 29±9 in females, p=0.13 and 23±19 vs.45±18, p=0.02 respectively).

Figure 2: Title- Extra cardiac manifestations

The non-cardiac manifestations in Danon patients are illustrated by gender and their prevalence in percent. Clinical myopathy, elevated creatine phosphokinase (>2 times normal limit), elevated liver enzymes and neurobehavioral problems (learning, neurological or psychiatric issues)) were more common in males than in females (p=0.001). The numbers above the bars refer to the absolute number of patients. CPK, creatine phosphokinase

Figure 3: Age of presentation and survival of Danon patients

Figure 3a: Title- Age of clinical presentation among LAMP2 mutation carriers

Figure shows cumulative percentage of carriers of pathogenic variants in LAMP2 who were clinically diagnosed with the disease (i.e. Danon disease) against age and and according to gender. Two groups are depicted: blue and green lines correspond to patients from this project, whereas yellow and red lines belong to patients in Health in Code's database (which includes patients from the scientific literature as well as from

Health in Code's research). Both groups (Danon project and Health in Code's database) show similar age-dependence of diagnosis. Males are diagnosed at an earlier age, with up to 30% of male carriers being diagnosed with the disease during the first decade of life, whereas 30% of females are diagnosed from their mid-third decade of life onwards. All male patients received a clinical diagnosis of Danon disease in this project, whereas almost 20% of females remained undiagnosed at last available follow-up.

Figure 3b: Title- Survival free of cardiovascular death in Danon disease patients

Figure shows the Kaplan-Meier survival curve free from cardiovascular death in carriers of pathogenic variants in LAMP2 belonging to this research (Danon project) compared with findings from Health in Code's database. Both groups are divided according to gender. Male patients may present with events early in life, with a steep decline in survival from the second decade of life onwards. Female carriers present with events later in life, mainly from the fourth decade of life onwards.

See supplemental Figure 1 for percentage of events according to gender and age.

Supplement table 1: Title- Age of diagnosis and age of death by mutation type

Mutations found in this cohort include frameshift, nonsense, splicing and 1 major deletion. We compared mean age of diagnosis and death or terminal event between males and females by mutation type.

Terminal event, Death, assist device or heart transplant during the follow-up

Supplemental table 2: Title- Predictors of terminal events among the affected patients

We compared surviving patients with those who had a terminal events which include death, heart transplantation or left ventricular assist device implantation. Patients with

terminal event had reduced ejection fraction, significantly atrio-ventricular block (>1st degree AV block) and atrial fibrillation.

LV, left ventricular AV, Atrioventricular

Text tables:

Table 1: Geographic distribution of patients and families

Country	Families (n)	Patients (n)	Male	Female		Families with a history of SCD (n)
				Affected	Carrier	
Italy	13	18	10	8		5
Spain	5	11	3	7	1	1
Israel	1	9	4	2	3	1
Denmark	2	5	2	3		1
United Kingdom	2	4	3	0	1	1
Greece	2	4	3	1		1
France	2	3	2	0	1	1
Ireland	2	11	3	6	2	2
Total	29	65	30	27	8	13

SCD, sudden cardiac death

Table 2. Cardiac Manifestations among the affected patients

Characteristics	Males Affected (n=30)	Female Affected (n=27)	p
Age of diagnosis (Mean ± SD)	13±9	36±15	<0.001
Age of last follow up	19±12	38±16	<0.001
Cardiomyopathy – all (no, %)	28 (93%)	27 (100%)	0.49
Left ventricular wall thickness -no.(%)			0.49
No LVH	8 (27%)	7 (26%)	
LVH †	22 (73%)	20 (74%)	
LV thickness Mean ± SD (mm)	20±8	17±6	0.28
Left Ventricular function no. (%)			
Normal	18 (60%)	9 (33%) **	0.071
Hypokinetic *	12 (30%)	16 (59%)	0.06
Mean ± SD (mm)	34±11	28±13	0.05
Electrophysiological – no. (%)			
WPW	15 (50%)	11 (41%)	0.48
Atrioventricular Block			1
I	5 (17%)	4 (15%)	
II	2 (7%)	2 (7%)	
III	2 (7%)	2 (7%)	
Atrial Fibrillation	8 (27%)	15 (55%)	0.02
Sustained VT	6 (20%)	4 (15%)	0.73
VF / ARREST	4 (13%)	2 (7%)	0.67
Device Therapy – no. (%)			0.89
Permanent pacemaker	5 (17%)	7 (26%)	
ICD	11 (37%)	10 (37%)	
CRT-D	3 (10%)	2 (7%)	

† LVH, LV wall thickness (≥13 mm)
* Hypokinetic cardiomyopathy, LVEF<50%
# 2 patient expired prior to LVEF assessment

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Table 3. Regression analysis to characterize patients with a terminal event

Characteristics	Univariate OR	Univariate 95%CI	Univariate p	Multivariate OR	Multivariate 95%CI	Multivariate p
Age of diagnosis	1.02	0.99-1.06	0.16	NA	NA	NA
Atrial fibrillation	3.28	1.10-10.40	0.04	1.96	0.51-7.41	0.315
AV block >1st degree	9.33	1.50-181.54	0.04	6.52	0.89-135.74	0.109
Female gender	1.23	0.43-3.52	0.7	NA	NA	NA
Hypokinetic LV	5.56	1.85-18.24	0.003	3.85	1.06-14.96	0.043
LV thickness >13mm	2.07	0.61-7.70	0.25	NA	NA	NA

Adjusted regression analysis, potential covariates were analyzed in univariate logistic regression models, and variables found to have a $p < 0.01$ were included in the adjusted model.

Terminal event; Death, assist device or heart transplant during the follow-up

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Figures

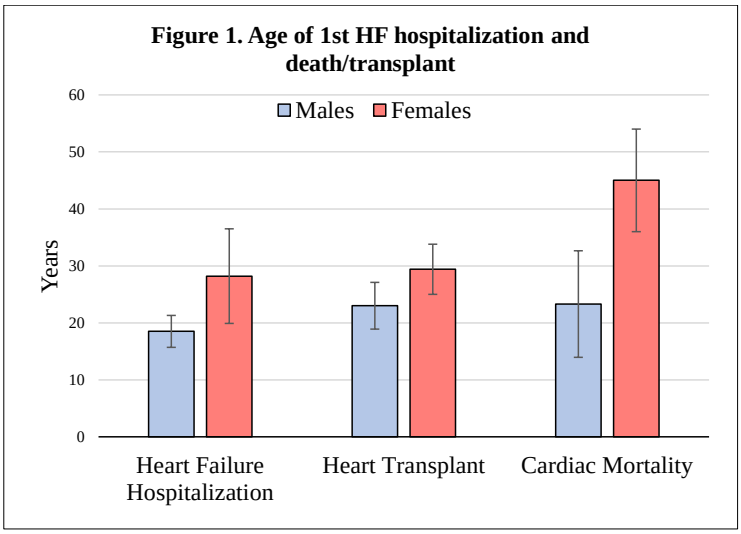


Figure 1. Age of 1st HF hospitalization and death/transplant
Males
Females
Years
60
50
40
30
20
10
0
Heart Failure Hospitalization
Heart Transplant
Cardiac Mortality

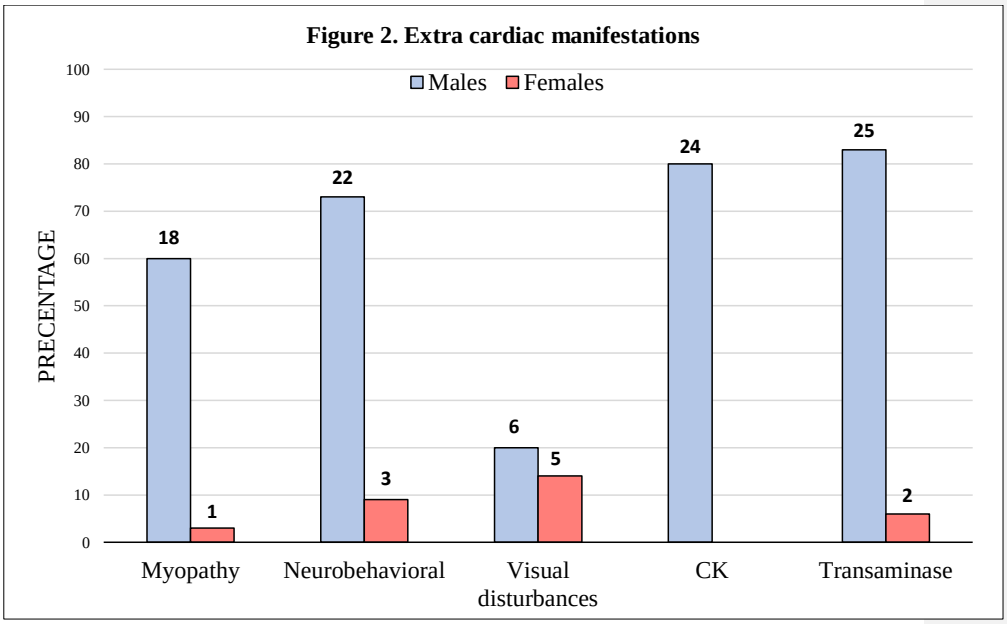


Figure 2. Extra cardiac manifestations
Males
Females
PRECENTAGE
100
90
80
70
60
50
40
30
20
10
0
18
1
22
3
6
5
24
25
2
Myopathy
Neurobehavioral
Visual disturbances
CK
Transaminase

Figure 3a: Age of clinical presentation among LAMP2 mutation carriers

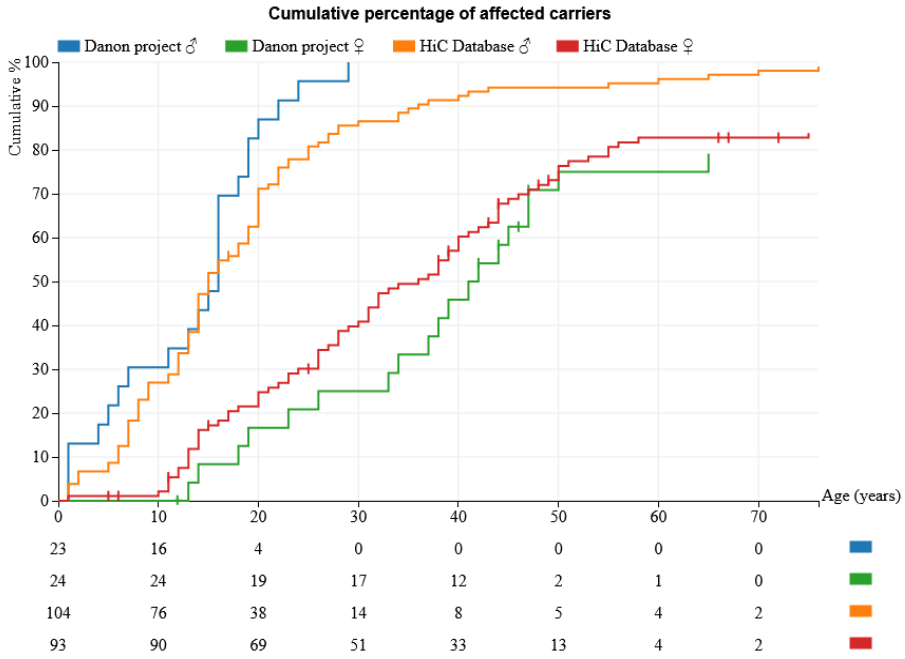
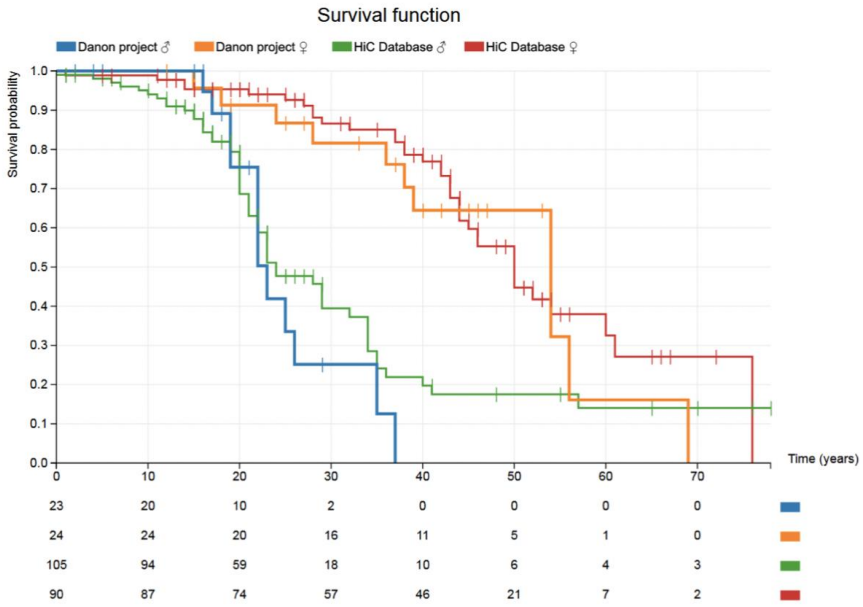


Figure 3b: Survival free of cardiovascular death in Danon disease patients



Supplemental table 2: Predictors of terminal events among the affected patients

Characteristics	Alive (n=29)	Terminal event* (n=28)	p
Age of diagnosis (years)	16.0 (6.0-36.0)	21.0 (16.0-41.2)	0.06
Male (no., %)	16 (55%)	14 (50%)	
Female (no., %)	13 (45%)	14 (50%)	0.696
Left ventricular hypertrophy (no., %)	20 (69%)	23 (82%)	0.358
LV thickness, >13mm (no., %)	20 (69%)	23 (82%)	0.358
Hypokinetic (LVEF<50%) (no., %)	9 (31%)	20 (71%)	0.002
Wolf parkinson white (no., %)	14 (48%)	12 (43%)	0.681
AV block, >1st degree (no., %)	1 (3%)	7 (25%)	0.025
Sustained ventricular tachycardia (no., %)	5 (17%)	5 (19%)	1
Atrial fibrillation (no., %)	8 (28%)	15 (56%)	0.034

*Terminal event: Death, Heart transplantation or left ventricular assist device implantation

Supplemental files

Supplemental table 1: Age of diagnosis and age of death by mutation type

Mutation type	Mutations (n)	Patients (n)	Age of diagnosis		Age of death or heart transplantation	
			Male	Female	Male	Female
Frameshift	9	13	9.9 (±10)	36.2 (±14.3)	17.8 (±3.3)	23.3 (±5.0)
Nonsense	8	18	9.8 (±8.8)	32.1 (±15.9)	23.0 (±8.4)	36.4 (±13.9)
Splicing	7	24	13 (±9.4)	35.2 (±13.4)	25.5 (±6.5)	44.1 (±18.1)
Major deletion	1	2	5	37		

Heart transplantation includes assist device implantation

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