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

Manuscript number: CIRCCVG/2020/003117-T2

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DOI: 10.1161/CIRCGEN.120.003117

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

Running title: *Lotan & Salazar-Mendiguchía et al.; Clinical Profile of Danon Cardiomyopathy*

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Journal Subject Terms: Genetics; Cardiomyopathy; Heart Failure; Hypertrophy

DOI: 10.1161/CIRCGEN.120.003117

Abstract:

Background - The X-linked Danon disease (DD) manifests by severe cardiomyopathy, myopathy, and neuropsychiatric problems. We designed this registry to generate a comprehensive picture of clinical presentations and outcome of patients with Danon disease (DD) in cardiomyopathy centers throughout Europe.

Methods - Clinical and genetic data were collected in 16 cardiology centers from 8 European countries.

Results - The cohort comprised 30 male and 27 female patients. The age at diagnosis was birth to 42 yr. in males and 2-65 in females. Cardiac involvement was observed in 96%. Extracardiac manifestations were prominent in males but not in females. Left ventricular (LV) hypertrophy was reported in 73% of male and 74% of female patients. LV systolic dysfunction was reported in 40% of males (who had LVEF 34±11%) and 59% of females (LVEF 28±13%). The risk of arrhythmia and heart failure (HF) were comparable among genders. The age of first HF hospitalization was lower in males (18 ± 6 vs. 28 ±17 yr., $p<0.003$). HF was the leading cause of death (10/17, 59%), and LV systolic dysfunction predicted an adverse outcome. Eight males and 8 females (28%) underwent heart transplantation or received a left ventricular assist device (LVAD). Our cohort suggests better prognosis of female compared to male heart transplant recipients.

Conclusions - DD presents earlier in males than females and runs a malignant course in both genders, due to cardiac complications. Cardiomyopathy features: heart failure and arrhythmia, are similar among the genders. Clinical diagnosis and management is extremely challenging in females due to phenotypic diversity and absence of extracardiac manifestations.

Key word: LAMP2; gender differences; cardiomyopathy

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Nonstandard Abbreviations and Acronyms

DD: Danon disease

Lysosome-Associated Membrane Protein 2: LAMP2

Left ventricular: LV

Ejection fraction: EF

Wolff Parkinson White: WPW

Implantable cardiac defibrillator: ICD

cardiac resynchronization therapy with an ICD: CRT-D

Left ventricular assist device: LVAD

Next-generation sequencing: NGS

Hypertrophic Cardiomyopathy: HCM

Introduction

Danon disease (DD) 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. DD, also termed Glycogen Storage Disease IIb, is caused by mutations in Lysosome-Associated Membrane Protein 2 (LAMP2). Given the role of LAMP2 in lysosomal fusion [3], LAMP2 deficiency leads to failure of the final step of the autophagic process, where digestion of aged cellular contents (including glycogen) and organelles takes place. The

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inability to remove aged mitochondria (mitophagy) leads to mitochondrial dysfunction, energetic deficiency, and oxidative stress [4–6]. Although DD is a multisystem disease, heart failure and arrythmia constitute the leading 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.

International registries are crucial in studying orphan diseases to accurately define the natural history, the outcome of interventions, and potentially enroll patients in therapeutic trials. We designed this registry to generate a comprehensive picture of the prevalence, clinical presentation, and outcome of DD patients, evaluated and followed in cardiomyopathy centers over Europe.

Methods

The authors declare that all supporting data are available within the article and available online as supplementary files. The study is a collaborative effort of a team of expert cardiologists across Europe who operate within a framework of specialized centers dedicated to cardiomyopathies and inherited heart diseases. Data were collected retrospectively using a consensus datasheet and interpreted by the primary investigators. Patient management and data collection were conducted according to local regulations, including an IRB approval for maintaining a Cardiomyopathy Registry, as required. Informed consent was obtained for all genetic studies performed for research or diagnostic purposes, as required. The full description of study methods and statistical analysis is available as supplemental data.

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Results

Epidemiology and Genetics

Thirty probands/families were collected by 15 participating centers from 8 European countries. Of the 65 patients included, 46% (N = 30) were affected males, and 54% (N = 35) females, including 27 affected and 8 gene carriers (Table 1).

There were 25 different mutations in the LAMP2 gene: 9 frameshifts, 8 affecting a splice-site, 7 nonsense variants and one major deletion predictive of complete gene absence (Suppl. Table 1). Among the clinically affected patients, the most common variants were mutations affecting a splice site (Suppl. Table 2). Mutations appeared to arise de novo in 13 probands. Prior family history of early cardiac death (prior to age 35 in males and 50 years in females) was reported in 12 (40%) families, representing a majority among those with familial disease. The age at diagnosis was significantly lower in males: mean 13±9 years (range birth to 42), compared to 36±14 in affected females (range 2-65, $p<0.01$, Figure 1). The mean age of female gene carriers was 31 years (range 2-46). There was no significant difference when comparing nonsense to frameshift and splice-site mutations for age of diagnosis, age of death or heart transplant (Suppl.Tab 2).

Clinical Manifestations

Cardiac involvement was seen in 55 of our patients (96%). Cardiomyopathy was present in all the affected individuals except two males (Table 2).

LV hypertrophy was reported in 73 % (n=22) of males, and 74% (n=20) of females with an average LV wall thickness of 20±8 and 17±6 (mm) respectively. Resting left ventricular gradient (defined as ≥ 30 mmHg) was reported in 4 (18%) males with LVH vs. none of the females (p=0.07). Hypokinetic cardiomyopathy was found at presentation in 40% (n=12) of

males and 59% (n=16) of females with mean EF of 34±11% and 28±13% respectively (p=NS). Left ventricular dilatation was reported in only 3 of these individuals. Forty-two patients (74%, 21 of each gender) reported symptoms of heart failure (NYHA II-IV).

Electrophysiological abnormalities such as Wolff Parkinson White (WPW) and early atria-ventricular block are considered indicators to suggest metabolic disease as possible cause of cardiomyopathy [7]. In our cohort, conduction abnormalities such as WPW pattern were reported in 15 (50%) males and 11 (41%) females, Table 2. Atria-ventricular block tended to be 1st degree. Advanced atria-ventricular block was reported in 15% (n=4) of 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).

Malignant ventricular tachyarrhythmia (sustained ventricular tachycardia, ventricular fibrillation, or cardiac arrest) occurred in both genders. A total of 38 (67%) patients received an implantable device (Table 2): permanent pacemaker, implantable cardiac defibrillator (ICD) or cardiac resynchronization therapy with an ICD (CRT-D). The most common (21/29) indication for ICD/CRT implantation was primary prevention. Eight were implanted for a secondary prevention after sustained ventricular tachycardia (n=4) or resuscitation for cardiac arrest due to ventricular fibrillation (n=4).

Extra-cardiac Features

Extracardiac manifestations in DD tend to aggravate morbidity and may constitute a clue to the diagnosis. Skeletal myopathy was clinically significant in 60% (n=18) of males but only in 3% (n=1) of females (p<0.001). Elevated creatine phosphokinase (>2 times normal limit) was reported in 80% (n=24) of males but not in women (p<0.001). Elevated liver transaminases enzymes were found in 83% (n=25) of males and 6% (n=2) of females (p<0.001). Pronounced

learning, neurological or psychiatric problems were quite common in males (n=22, 73%) but rare in females (n=3, 9%, p<0.001). Clinically manifest visual involvement was equally uncommon in both gender groups (Figure 2). Cumulatively, all males but only 10/27 females had at least 1 extracardiac manifestation (p<0.001).

Clinical course, outcome and causes of death

Fifty-three of fifty-seven (93%) clinically affected patients were followed up for at least one year, comprising a total of 350 patient-years. Fifteen males and 15 females (53% of the entire cohort) had a heart failure hospitalization, at mean age 18 ± 6 compared to 34 ±13 years, p<0.003, Figure 1). Sixteen (27%) underwent heart transplantation or received a left ventricular assist device (LVAD). Eight were males (6 heart transplantations and 2 had LVAD implanted), 8 were heart transplanted females (mean ages 23±8 and 29±9 years respectively, p=0.13,). Seventeen patients (30% of the cohort, 11 males and 6 females) died. The mean age of death was 23±19 years among men, and 45±18 in females (p=0.02, Figure 1). The ages and causes of death are presented in Table 3.

The leading cause of death was heart failure, which was reported in 10 patients (59%). Five of the 8-heart transplant/assist device male recipients (62%) died within one year from the procedure. Causes of death after heart/LVAD transplantation were variable and include heart failure, rejection, stroke, and an early post-transplant non-Danon related death. No death was reported among females receiving a heart transplant during a mean follow up of 10.6±5.6 years (range 4-17).

Sudden cardiac death occurred in 3 female patients and was the first presentation of DD in one of them (Figure 3). Appropriate ICD/CRTD intervention for sustained ventricular

arrhythmia (VT or VF) was reported in 6 patients (3 of each gender), 4 of whom were implanted for primary prevention (Table 2).

In a univariate analysis of factors determining prognosis, patients who had a terminal event (death, assist device or heart transplantation) were older at diagnosis and at the time of initial evaluation (Table 4 and suppl. Table 3). Reduced left ventricular systolic function was also 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 included variables that were significant in univariate analysis, only a hypokinetic left ventricle remained a significant predictor of mortality. This result did not change with the inclusion of age and gender in the multivariate model (OR 3.85, 95%CI 1.06-14.96, p=0.043, Table 4).

Disease Expression and Survival Curves

Cumulative incidence of disease expression and survival by age was calculated and illustrated in Figure 4. Comparing our cohort to all DD cases reported in the literature (comprising data from 105 males and 90 females) showed no notable difference between the two cohorts, thereby demonstrating that our cohort is highly representative of both the male and female population with DD .

Discussion

The results of this European multicenter registry show that DD is associated with severe cardiac morbidity and mortality in both men and women. Our data on 57 genetically and clinically affected patients, evaluated in Cardiomyopathy centers over Europe demonstrates a high expression and rapid progression of cardiac disease both in men and women. There were

pronounced differences among genders in extra-cardiac manifestations and the age of presentation but not in the specific features of the cardiac phenotype or complications (Table 2). A majority developed heart failure close to the time of diagnosis, often leading to heart transplantation or death (Figure 1, Table 3 and Suppl. Table 2).

DD is a lysosomal storage disease, caused by LAMP-2 dysfunction or deficiency, that is transmitted as X-Linked with a dominant inheritance trait. 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[8]. The presence of a normal allele may also account for the near absence of extracardiac manifestations in females. Although different gene defects may have various effects on splicing and protein expression, we did not find major differences in disease presentation among the mutation types regarding the age of presentation by gender or complications (Suppl. Tables 1,2). The high proportion of de novo mutations should be viewed as another marker of severe early-onset disease adversely affecting reproduction.

Diagnosis of DD is very uncommon even in cardiomyopathy clinics and may not be familiar to many general cardiologists. The real prevalence in population is unknown. Widespread screening using multiple parallel /next-generation sequencing (NGS) led to the detection of LAMP-2 mutations among cardiomyopathy and myopathy patients implying a unique (often unexpected) diagnosis with grave medical and therapeutically consequences [9,10].

Highly selected cohorts from referral centers have shown disease prevalence 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 as high as 6-8% in adults with concentric HCM [15], 17-30% in patients with HCM and pre-excitation on ECG [13,16,17], and even 33% among pediatric patients

with HCM and vacuolar myopathy[14,18,19] . DD was reported in women with cardiomyopathy, including those with early conduction system disease or a postpartum presentation. In contrast, no Danon disease patients were identified by NGS screening of 72 children with idiopathic dilated cardiomyopathy [11]. NGS studies of 17704 index cases that included more than 10000 probands with cardiomyopathy, identified only 18 pathogenic or likely pathogenic LAMP2 variants (personal communication, Lorenzo Monserrat, Health in Code laboratory, Spain). Differences in the availability and applicability of gene testing in individuals with cardiomyopathy, i.e. routine gene testing for all HCM/DCM vs. restricting gene testing to unique phenotypes (such as like cardiomyopathy with extracardiac features), could have the following impacts: 1) Number of DD cases reported by cardiomyopathy experts from different countries (Table 1); 2) Variability of age and disease stage when diagnosis is made in females with DD (Fig. 1, Suppl. Table 2).

Data based on case reports and single-center studies may not represent the possible heterogeneity between families and ethnicities across the globe. The problem was addressed by multicenter and web-based registries. López-Sainz et al. recently reported on 27 patients, mainly women, collected from all over Spain [20]. Sugie et al. [21,22] collected a total of 77 Japanese patients. Boucek et al [23] reported 82 patients widely dispersed across four continents, of whom only 44 had clinical findings. Cenacchi et al.[19] and recently reviewed all 332 published cases of DD to date, based on case reports, small series, as well as 186 patients from large series. Brambatti et al[24] identified 56 females and 90 males with DD through MEDLINE and EMBASE search. These studies primarily differ in their estimates of cardiac complications, and the mode of cardiac presentation (i.e. HCM vs. DCM), in particular among women, as well as the prevalence of various electrophysiological and extracardiac features.

In our study, performed in dedicated cardiomyopathy clinics, a comparable proportion of females and males presented with LVH and/or systolic dysfunction. Furthermore, the wall thickness and the EF were similar in both genders. This finding contradicts previous analyses which suggested that females present more with DCM compared to massive HCM in males [19]. A comprehensive recent literature review[24] reports that 96% of males and 70% of females present with HCM. Hypokinetic cardiomyopathy associated with wall thinning and tends to develop in both genders during the course of disease progression (10, 21). The discrepancy between the reports may arise from the different time points of evaluation and different approaches to gene testing. There might be a greater recognition of the disease manifestations at its different stages, primarily in females, due to proactive screening in dedicated centers,

Females had a similar prevalence of life threatening ventricular arrhythmia, but more atrial fibrillation, similar to smaller series reported previously [25]. Unexpected sudden cardiac death in 3 female patients (comprising 50% of mortality among women in this cohort) alarmingly demonstrates their arrhythmic risk, which may be only partly predicted by massive hypertrophy or reduced EF (Tables 2,3).

Extra-cardiac manifestations were prominent in males but uncommon in females, making the clinical diagnosis in affected women (in the absence of family history or genetic testing) extremely challenging. In-depth investigations of visual and psycho-cognitive testing may disclose subtle abnormalities in the affected females. IQ and cognitive abilities assessment by standardized cognitive measurements, did not demonstrate intellectual disability/mental retardation but rather mild (if any) cognitive and psychological deficits [11,26]. Yet, those are rarely sufficiently pronounced to draw attention in the early stages of the disease (Figure 2). These

findings emphasize the importance of gene testing in women presenting with *idiopathic* cardiomyopathy and require inclusion of LAMP2 in gene panels used in routine clinical practice.

Reduced left ventricular systolic function was the only independent predictor of prognosis in our study. This finding corresponds to progressive myocardial fibrosis which is the hallmark of cardiac Danon disease, leading to heart failure evoked by combined systolic and diastolic dysfunction (Figure 3). The same pathological substrate also accounts for arrhythmic propensity which may manifest as sudden death either at presentation or as a complication of advanced heart failure [10]. While novel therapies are being developed, contemporary therapies to attenuate fibrosis might be useful to modify the course of cardiomyopathy [27,28].

Five of 8 male transplant/LVAD recipients died within a year after the procedure which indicates potential problems with heart transplantation in males with DD. With few remaining options, the transplant clinic should be prepared to deal with neuromuscular complications (e.g., post-transplant myopathy) and psychiatric issues that may occur in the peri- and postoperative period. While there are numerous case reports of successful heart transplantation in DD males, cases with adverse outcomes are less likely to be reported. This issue needs to be addressed in a prospective multicenter registry [29,30].

Hopefully our findings will stimulate prospective observational studies incorporating objective functional assessment and CMR data to explore the critical turning points throughout the course of the disease. While no specific therapy is available, attempts to develop gene therapy or (possibly) protein therapy are underway [29]. Lifestyle intervention and conventional therapies should be assessed to optimize patient care and disease management[30].

DOI: 10.1161/CIRCGEN.120.003117

Limitations and perspectives

This is an observational cohort dealing with a lethal orphan disease. Given the retrospective nature of our study, we had difficulty in following disease progression as well as systematically adjudicating arrhythmic events. DD heart is characterized by progressive fibrosis leading to heart failure. Due to limitations imposed by data collection as well as restrictions by disease, in most patients did not have neither magnetic resonance imaging data or functional testing to assess their relationship to clinical events and outcomes.

However, these were inevitable consequences of the very low prevalence of DD. Nevertheless, the rapid progression of the disease and high number of events in our study cohort, combined with a relative long period of follow-up, allowed us to obtain novel and important evidence regarding the clinical presentation and natural history of DD in males and females.

Conclusions

DD expresses earlier in males than females but runs a highly malignant course in both genders, mostly related to HF complications. Cardiomyopathy features: heart failure and arrhythmia, are similar among the genders. Clinical diagnosis and management is extremely challenging in females due to phenotypic diversity and absence of extracardiac manifestations.

Early diagnosis combined with understanding the clinical spectrum and natural history, are essential to optimize the care of patients and families with DD. Treatment must address disease progression and implement strategies for sudden death prevention.

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Sources of Funding: This work was partially supported by grants from the "Instituto de Salud Carlos III", FEDER "European Union, Una forma de hacer Europa" La Fe Biobank [PT17/0015/0043] and Memorial Nacho Barberá**.** Philippe Charron is supported by "Determination of the role of clinical and epigenetic risk markers in dilated cardiomyopathy and heart failure" (DETECTIN-HF) project of European Research Area Network on Cardiovascular Diseases (ERA-CVD); and the PROMEX charitable foundation. Lorenzo Monserrat is share-holder in Health in Code S.L.

Disclosures: none to disclose

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DOI: 10.1161/CIRCGEN.120.003117

Table 1: Geographic distribution of patients and families

Country	Families (n)	Patients (n)	Male	Female		Families with a history of early cardiac death (n)
				Affected	Carrier	
Italy	14	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		0
France	2	3	2	0	1	1
Ireland	2	11	3	6	2	2
Total	30	65	30	27	8	12

The distribution of Danon disease patients by countries involved in our registry, number of families and patients with gender differentiation. Last column refers to number of families per country with a history of early cardiac death (defined by age <35 years in males and <50 in females) .

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Table 2: Cardiac manifestations at the time of evaluation and devices implanted among the affected Danon patients are presented by gender.

Characteristics	Males Affected (n=30)	Female Affected (n=27)	p
Age of Diagnosis (yr, mean ± SD, range)	13±9 (1-42)	36±15 (13-65)	<0.001
NYHA Class at Evaluation, n (%) *			1
Class I-II	19 (63%)	17 (63%)	
Class III-IV	7 (23%)	7 (26%)	
Age of last follow up (yr, mean ± SD, range)	19±12 (2-38)	38±16 (18-69)	<0.001
Cardiomayopathy – all (no, %)	28 (93%)	27 (100%)	0.49
Left ventricular wall thickness			0.49
No LVH (n, %)	8 (27%)	7 (26%)	
LVH † (n, %)	22 (73%)	20 (74%)	
LV thickness (Mean ± SD (mm), range)	20±8 (13-39)	17±6 (13-35)	0.28
Left Ventricular function no. (%)			
Normal (n, %)	18 (60%)	9 (33%) ‡	0.071
Hypokinetic # (n, %)	12 (30%)	16 (59%)	0.06
LVEF % (Mean ± SD, range)	34±11 (16-50)	28±13 (15-50)	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
ICD	12	12	
Pacemaker	4	5	
CRT-D	3 (10%)	2 (7%)	

* 4 Males, and 2 Females NYHA class was missing.
† LVH, LV wall thickness (≥13 mm)
‡ 2 patient died before LVEF assesment
# Hypokinetic cardiomyopathy, LVEF<50%
LVH, Left ventricular hypertrophy; NYHA, New York Heart Association; WPW, Wolff Parkinson White; VT, Ventricular tachycardia; VF, Ventricular fibrillation; ICD, Implantable cardioverter-defibrillator; CRTD, cardiac resynchronization therapy with defibrillator. Data on arrhythmia constitutes a summary of baseline and follow up reports.

DOI: 10.1161/CIRCGEN.120.003117

Table 3: The ages, main clinical features, interventions and causes of death in DD patients who expired

Subject	Gender	Age of diagnosis	LV wall thickness	LVEF	Arrhythmia	Device	HTx / LVAD	Age of death	Cause of death
1	m	14	35	70	WPW, I AVB	ICD		17	Heart failure
2	m	7	35	70	CAF	ICD		22	Heart failure
3	m	7	14	37	VT	PPM	HTx age 14	14	Heart Failure
4	m	16	18	25	WPW,CAF		HTx age 16	16	Organ rejection
5	m	19	25	50	WPW,III AVB,VT	ICD		26	Heart Failure and VF
6	m	19	25	45	WPW,CAF,VT	ICD		23	Heart failure
7	m	24	16	20	II AVB,VT	ICD		25	Heart failure
8	m	22	16	16	WPW,II AVB,CAF	PPM	HTx age 22	22	Non-Danon related death 7 days after HTx
9	m	20	15	55	III AVB	CRT-D	LVAD age 35	35	Cerebrovascular event in context of ventricular assist device
10	m	11	7	35	WPW,I AVB,CAF	CRT-D		19	Heart failure
11	m	38	12	28	CAF, I AVB	CRT-D	HTx age 38	38	Low CO state, death 9 days after a heart transplant.
12	f	65	13	35	III AVB,CAF	CRT-D		69	Heart failure
13	f	26	15	NA	none			28	Sudden cardiac death
14	f	44	13	30	WPW,CAF,VT	ICD		54	Heart failure
15	f	18	35	NA	NA			18	Sudden cardiac death
16	f	46	7	15	CAF	ICD		54	Heart failure
17	f	47	14	25	III AVB	PPM		47	Sudden cardiac death

LVEF, left ventricular ejection fraction; BiVN, biventricular hypertrophy on autopsy; WPW, Wolff Parkinson White; AVB, atrioventricular block (degree); CAF, chronic atrial fibrillation; VT, ventricular tachycardia; VF, ventricular fibrillation; ICD- implantable cardioverter-defibrillator, PPM- permanent pacemaker, HTx- heart transplantion, CRT-D- implantable cardiac resynchronization therapy with a defibrillator, LVAD- left ventricular assist device; CO, cardiac output; NA, non available.

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Table 4. 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

The table presents the results of logistic regression analysis. Potential variables were analyzed in univariate logistic regression models. Variables found to be significant at $p < 0.1$ were included in the multivariate model adjusted for age of diagnosis and gender.
Terminal event, Death, implantation of an assist device or heart transplantion during the follow-up; NA, non-applicable.

DOI: 10.1161/CIRCGEN.120.003117

Figure Legends:

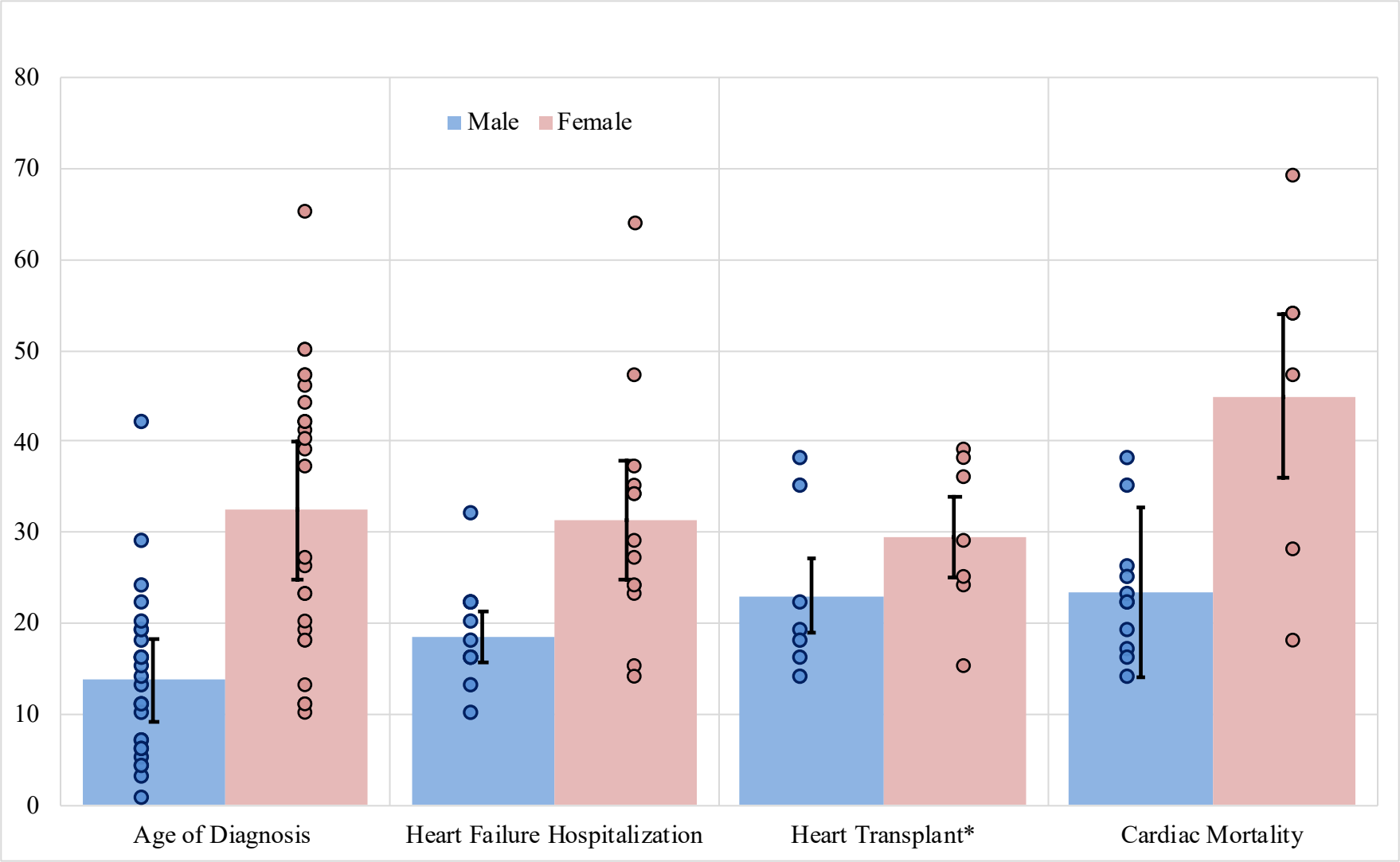
Figure 1: Age at diagnosis and heart failure events. Mean age (±SD) of diagnosis, first heart failure hospitalization, heart transplantation* or death, stratified by gender. Dots represent individual patients with events. The age of diagnosis and of first heart failure hospitalization was higher in females ($p<0.01$) and so was their age of death ($p=0.02$). On the contrary, the mean age at heart transplantation was not significantly different ($p=0.13$). *, left ventricular assist device included.

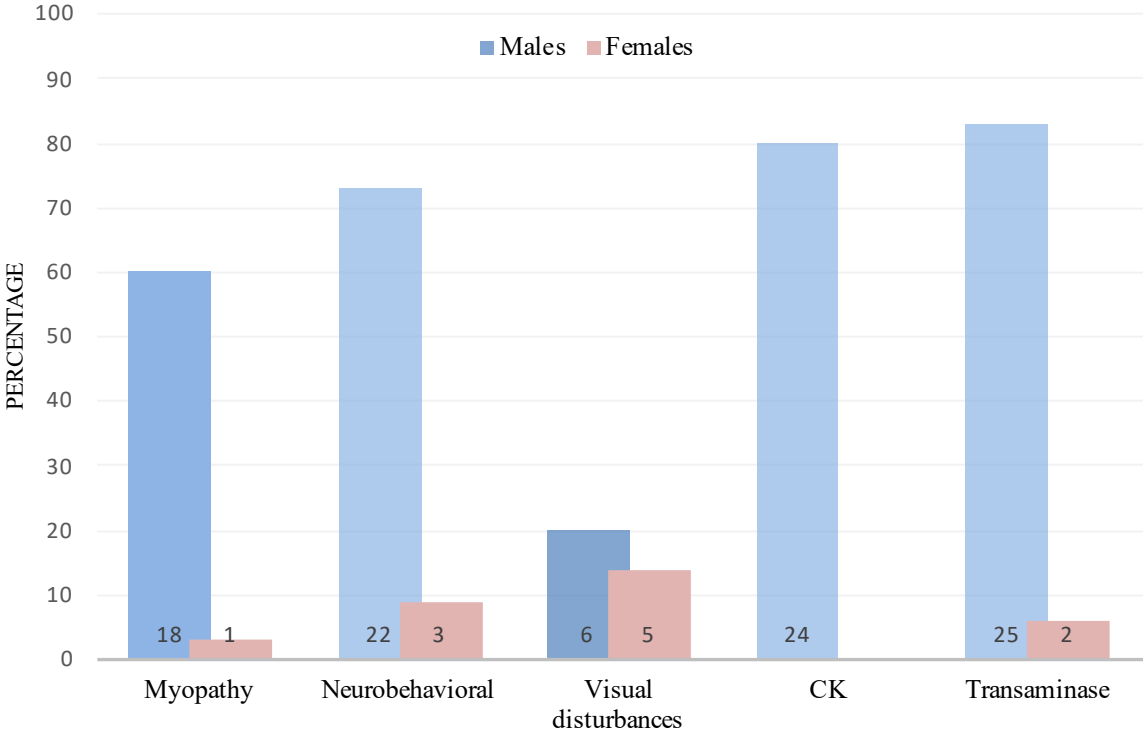
Figure 2: Title- Extra-cardiac manifestations. The non-cardiac manifestations in Danon patients are illustrated by gender and their prevalence in percent. Clinical myopathy, elevated creatine phosphokinase (>2 times normal limit), elevated liver enzymes, and neurobehavioral problems (learning, neurological,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

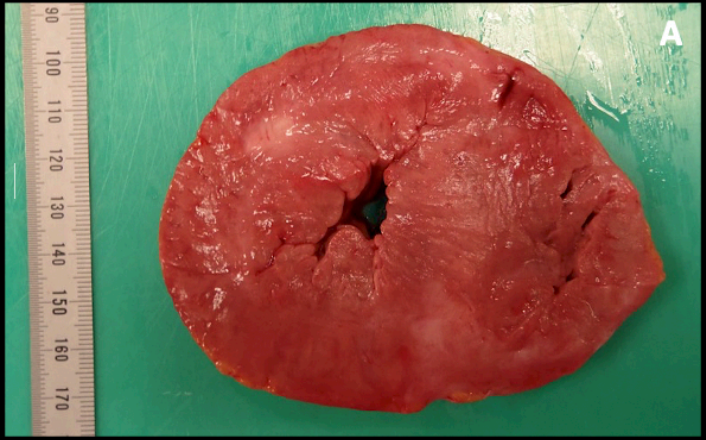
Figure 3: Autopsy findings of a teenage girl who died suddenly with a PM diagnosis of DD. **A, B**: Autopsy from a 18 years old female (#15 in Table 3, M2 in supplementary Table 1) died suddenly. She was suffering from a mild effort limitation but was not previously evaluated. The diagnosis of Danon disease was confirmed by identifying a c.1093del *LAMP2* mutation predicting early frameshift and early termination (p.Ala365Leu fs*15) of protein. A) Gross examination shows massive hypertrophy with maximal wall thickness of 35 mm. B) Masson Trichrome stain demonstrates fibrosis replacing most of the myocardial cross-section and scattered vacuolated cardiomyocytes (courtesy of Prof. Ulrik Baandrup, DK). **C, D**: CMR from an asymptomatic 17 years old male (DD1 family, Suppl. Table 1) showing

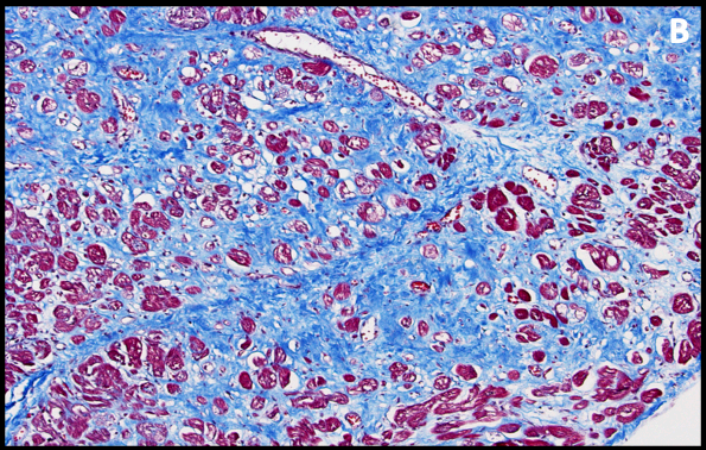
extensive late gadolinium enhancement compatible with myocardial fibrosis in short axis oblique (C) and 2 chamber view (D). There was concentric hypertrophy with maximal wall thickness of 18 mm, and LVEF 68%.

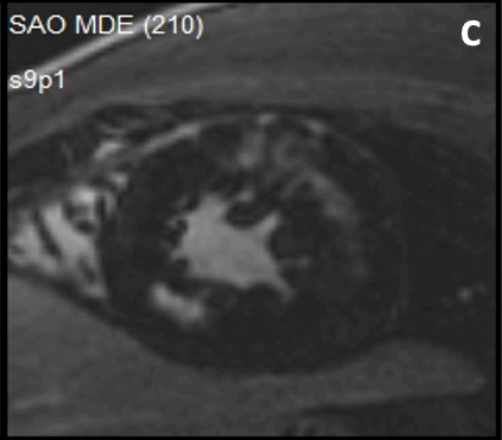
Figure 4: Age of presentation and survival of Danon patients. **A**: Title- Age of clinical presentation among LAMP2 mutation carriers. Figure shows the cumulative percentage of carriers of pathogenic variants in LAMP2 who were clinically diagnosed with the disease (i.e., Danon disease) against age and 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 female mutation carriers remained asymptomatic at the last available follow-up. **B:** 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 present with events early in life, with a steep decline in survival from the second decade of life onwards. Females suffer from disease complications later in life, mainly from the fourth decade of life onwards.

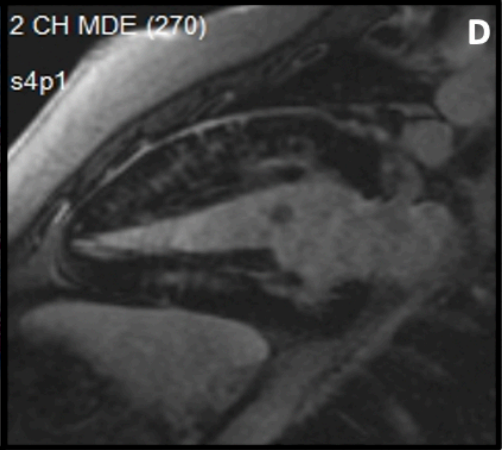

80
70
60
50
40
30
20
10
0
Male
Female
Age of Diagnosis
Heart Failure Hospitalization
Heart Transplant*
Cardiac Mortality

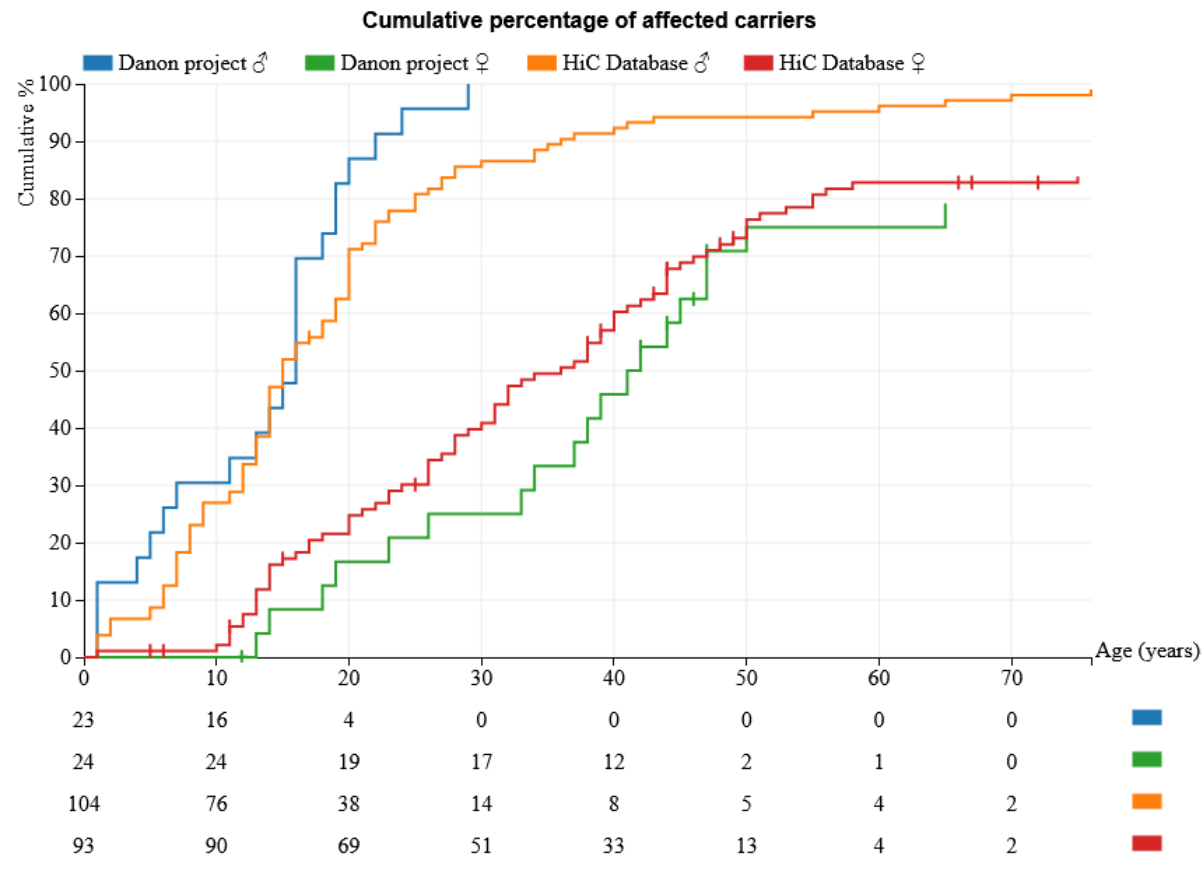

100
90
80
70
60
50
40
30
20
10
0
PERCENTAGE
Males
Females
18
1
22
3
6
5
24
25
2
Myopathy
Neurobehavioral
Visual disturbances
CK
Transaminase

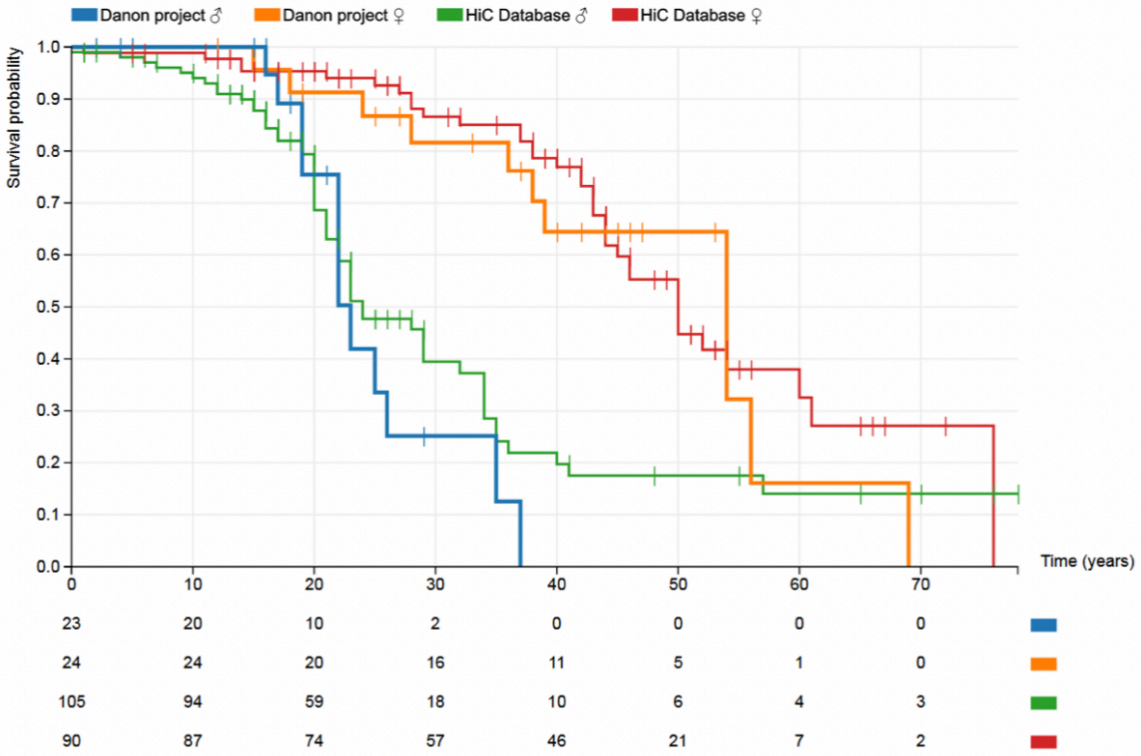

A


B


SAO MDE (210)
s9p1
C


2 CH MDE (270)
s4p1
D


Cumulative percentage of affected carriers
Danon project ♂
Danon project ♀
HiC Database ♂
HiC Database ♀
Cumulative %
100
90
80
70
60
50
40
30
20
10
0
Age (years)
0
10
20
30
40
50
60
70
23 16 4 0 0 0 0 0
24 24 19 17 12 2 1 0
104 76 38 14 8 5 4 2
93 90 69 51 33 13 4 2


Survival function
Danon project ♂
Danon project ♀
HiC Database ♂
HiC Database ♀
Survival probability
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
10
20
30
40
50
60
70
Time (years)
23 20 10 2 0 0 0 0
24 24 20 16 11 5 1 0
105 94 59 18 10 6 4 3
90 87 74 57 46 21 7 2