Clinical Characteristics and Natural History of Dilated Cardiomyopathy due to BLC2-associated Athanogene 3 (*BAG3)* Mutations

Short Title: DCM caused by *BAG3* mutations.

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Abstract:

Background: The *BAG3* (BLC2-associated athanogene 3 (BAG3) gene codes for an anti-apoptotic protein located on the sarcomere Z-disc. Mutations in *BAG3* are associated with dilated cardiomyopathy (DCM), but only a small number of cases have been reported to date and the natural history of *BAG3* cardiomyopathy is poorly understood

Objectives: This study sought to describe the phenotype and prognosis of *BAG3* mutations in a large multicenter DCM cohort.

Methods: The study cohort comprised 129 individuals with a *BAG3* mutation (62% males, 35.1±15.0 years) followed at 18 European centers. Localization of BAG3 in cardiac tissue was analyzed in patients with truncating *BAG3* mutations using immunohistochemistry.

Results: At first evaluation, 57.4% of patients had DCM. After a median follow-up of 38 months (interquartile range: 7–95), 68.4% of patients had DCM and 26.1% who were initially phenotype-negative developed DCM. Disease penetrance in individuals aged >40 years was 80% at last evaluation, and there was a trend towards an earlier onset of DCM in men (34.6±13.2 vs 40.7±12.2; p=0.053). The incidence of adverse cardiac events (death, left ventricular assist device, heart transplantation, and sustained ventricular arrhythmia) was 5.1% per year among individuals with DCM. Male sex, decreased left ventricular ejection fraction (LVEF) and increased left ventricular end-diastolic diameter (LVEDD) were associated with adverse cardiac events. Myocardial

tissue from patients with a *BAG3* mutation showed myofibril disarray and a relocation of BAG3 protein in the sarcomeric Z-disc.

Conclusions: DCM caused by mutations in *BAG3* is characterized by high penetrance in carriers over 40 years and a high risk of progressive heart failure. Male sex, decreased LVEF and enlarged LVEDD are associated with adverse outcomes in patients with *BAG3* mutations.

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Keywords: Genetics, Dilated cardiomyopathy, Prognosis, *BAG3*.

Condensed abstract:

BAG3 mutations are associated with dilated cardiomyopathy (DCM), but only a small number of cases have been reported. This study describes the phenotype and natural history of 129 individuals with a *BAG3* mutation. The prevalence of DCM in *BAG3*-mutated individuals older than 40 years of age was 80%, and there was a trend towards an earlier DCM onset in males (p=0.053). Incidence of adverse cardiac events was 5.1% per year in patients with DCM, with a predominance of heart failure complications. Male sex, decreased left ventricular ejection fraction and enlarged left ventricular end-diastolic diameter were associated with adverse outcomes.

Condensed abstract word count: 99

Abbreviations:

BAG3: BCL2-associated athanogene 3

CK: Creatine kinase

DCM: Dilated cardiomyopathy

HF: Heart failure

HSP: Heat shock proteins

HTx: Heart transplantation

ICD: Implantable cardioverter defibrillator

INDEL: insertion/deletion

IQR: Interquartile range

LMNA: Lamin AC

LVED: Left ventricular end-diastolic diameter

LVEF: Left ventricular ejection fraction

LVAD: Left ventricular assist device

NSVT: Non-sustained ventricular tachycardia

NYHA: New York Heart Association

SCD: Sudden cardiac death

SVT: Sustained ventricular tachycardia

TTN: Titin

VF: Ventricular fibrillation

Introduction

Dilated cardiomyopathy (DCM) is defined as dilation and systolic impairment of the left ventricle that is not attributable to abnormal loading conditions or coronary artery disease. It is the most common cause of heart failure in the young and the most frequent indication for heart transplantation (HTx) (1). Recent studies suggest that up to 50% of patients with DCM have a genetic predisposition to the disease caused by mutations in more than 60 genes (2,3), many described very recently (4–6).

One of the recent genes of interest is *BAG3* (7,8), which encodes for BLC2-associated athanogene 3 (7,8) (BAG3), a co-chaperone that interacts with members of the heat shock protein (HSP) family (9). These highly conserved proteins are released by the cell in response to stress, and their role is to stimulate the repair and degradation of protein aggregates that accumulate under stress situations such as induced tension in striated muscle cells (10). Moreover, it has been shown that BAG3 is essential for the normal production and clearance of filamin (10) and modulates myocyte contraction through interaction with the β1-adrenegic receptor and the L-type calcium channel (11). Heterozygous *BAG3* mutations in human induced pluripotent stem cell-derived cardiomyocytes have been shown to disrupt the myofibril structure and compromise contractile function (12).

Since its initial description as a DCM-causing gene, several cases of DCM caused by mutations in *BAG3* have been described, and the prevalence of *BAG3* DCM has been reported to be between 2.3% and 3.6% in DCM patient cohorts from the United States, Europe and Japan (7,8,13). Nevertheless, thus far all published reports are mostly unicentric, include a limited number of individuals, and provide limited information on cardiac outcomes (14,15). Furthermore, and despite the fact that *BAG3* mutations have

been associated predominantly with DCM, a missense mutation in *BAG3* (p.Pro209Leu) has been associated with hypertrophic and restrictive cardiomyopathy, and also myofibrillary myopathy (16).

In this multicenter retrospective study, we sought to determine the mode of presentation and long-term outcomes of DCM patients and asymptomatic relatives with pathogenic *BAG3* mutations. Furthermore, in an effort to gain insight into the consequences of *BAG3* mutations at the cardiomyocyte level, we examined by immunohistochemistry the localization of BAG3 in cardiac tissue obtained from DCM patients with truncating *BAG3* mutations.

Methods

A chart review was performed in consecutive probands and evaluated relatives with pathogenic or likely pathogenic *BAG3* mutations followed at 18 European centers (Online material). Clinical, electrocardiographic (resting 12-lead and ambulatory electrocardiogram [ECG] monitoring) and echocardiographic data from the first and last medical contact at each center were recorded. Left ventricular dysfunction was defined as a left ventricular ejection fraction (LVEF) ≤50% (17).

Details of clinical events that had occurred before first clinical contact and during follow-up (including the timing of events) were collected. Events were characterized as follows: left ventricular assist device (LVAD) implantation, heart transplantation (HTx), sustained ventricular tachycardia (SVT), successfully resuscitated ventricular fibrillation (VF), appropriate implantable cardioverter defibrillator (ICD) shock, sudden

cardiac death (SCD), and cardiac and all-cause mortality. SCD was defined as an unexpected death due to cardiac causes that occur within one hour of the onset of symptoms.

The composite of SVT, VF, ICD shock and SCD was categorized as a serious arrhythmic event, whereas the composite of HTx, LVAD implantation and heart failure (HF) death was categorized as an HF-related event. The primary endpoint of adverse cardiac events was defined as a composite of serious arrhythmic events and HF-related events.

Genetic analysis and classification of variants

Deoxyribonucleic acid (DNA) sequence analysis was performed at the participating institutions. Variants were categorized as truncating (nonsense, frameshift, copy number variations [CNV] and splice site variants) or non-truncating (missense, small insertion/deletion). Pathogenicity of variants was established according to the current American College of Medical Genetics and Genomics guidelines (18). Novel sequence variants (not found in controls) that predicted a premature truncation were also considered pathogenic.

Inmunohistochemistry

Paraffin-embedded sections from explanted heart tissue samples were stained with BAG3 (Abcam ab 47124, Cambridge, UK) and sarcomeric α-actinin (Abcam ab 9465) antibodies, allowing visualization of the sarcomeric architecture and the localization of BAG3 in the cell. A detailed description of the tissue processing protocol can be found in Supplemental material (Online material).

Heart tissue was studied in 3 DCM patients with pathogenic mutations in *BAG3* (p.Ala128Glufs*84, p.Val439Glyfs*4 and p.Arg301Serfs*6), 2 DCM patients with truncation mutations in the titin gene (*TTN*) (Pro19967Leufs*8, Arg18745Leufs*69), 1 DCM patient with a pathogenic mutation in the lamin gene (*LMNA*) (p.Arg321*) and 2 DCM patients without identified mutations in DCM-causing genes.

Statistical analysis

Results are presented as mean ± standard deviation for continuous variables with normal distribution, as median and interquartile range (IQR) for continuous variables without normal distribution, and as number (percentage) for categorical data. For statistical analysis, Student´s t test and the Mann-Whitney nonparametric test were used in 2-group comparisons, whereas analysis of variance and Tukey test for multiple group comparisons were applied for 3 groups. Chi-square test or Fisher´s exact test were used for categorical variables. The cumulative probability of the occurrence of serious adverse cardiac events was estimated using the Kaplan-Meier method, and factors were compared using the log rank (Mantel-Cox) method. Survival was calculated from first evaluation. A 2-sided p-value <0.05 was considered statistically significant. Statistical analyses were performed using SPSS Statistics version 21.0 (IBM, Armonk, New York).

Results

The study cohort included 129 individuals (62% males, 35.1±15.0 years at first evaluation) from 38 families (median 2 subjects per family). All individuals were Caucasians of European ancestry with the exception of one male Asian subject from Afghanistan with the p.Arg309* variant. Most subjects carried truncating mutations (86%). The complete list of mutations is available in Supplemental material (Table S1).

At first evaluation, 74 patients had DCM while 55 subjects had normal left ventricular size and function (Table 1, Figure 1). After a median follow-up of 38 months (IQR: 7–95), 78 patients had DCM (78/114; 68.4%), 36 (36/114; 32.6%) showed normal phenotype and 15 (6 with DCM) were lost to follow-up (15/129; 11.6%). A total of 12 patients without DCM at initial evaluation developed DCM (12/46 with available follow-up; 26.1%), 2 patients with DCM at initial evaluation normalized LVEF (2/68 with available follow-up; 2.9%) and 80% (48/60) of individuals older than 40 years at last evaluation exhibited DCM (Figure 1). A total of 18 DCM patients had been transplanted (n=17) or underwent LVAD implantation (n=1) at last evaluation (18/78; 23%) (Figure 1). Overall and cardiac mortality during follow-up in patients with DCM was 10.3% (8/78) and 8.9% (7/78), respectively.

Natural history of BAG3 mutations

The diagnosis of DCM was made at a mean age of 36.9±13.1 years, with a trend towards an earlier onset in men (34.6±13.2 vs 40.7±12.2; p=0.053). The type of mutation (truncating vs non-truncating) had no impact on the mean age at DCM diagnosis (37.3±12.7 in truncating vs 35.5±14.5 years in non-truncating; p=0.62). A total of 12 individuals (83.3% male) were diagnosed with DCM under 20 years of age (12/71 patients with available information; 17%); a 14 year-old male with a non-sense

mutation (p.Gln353Argfs*10) was the youngest patient diagnosed. Seven-percent of patients were diagnosed with DCM over 55 years.

Among patients with DCM at first evaluation (n=74), 24 (32.4%) were in NYHA class I, 18 (24.4%) were in NYHA class II, and 32 (43.2%) were in NYHA class III-IV. ECG and echocardiographic findings at first evaluation are shown in Table 1. Patients with DCM at first evaluation were in sinus rhythm in 95.6% of cases, 22% showed negative T waves and the mean QRS duration was 99.4±20.3 ms. Mean ejection fraction was 32.9±13.1% and the mean LV end-diastolic diameter (LVEDD) was 64.3±7.7 mm. Mutation carriers without DCM phenotype were all in sinus rhythm and 16% exhibited negative T waves in the ECG (Table 1).

Of the 12 patients who developed DCM during follow-up (38.5% males, age at DCM diagnosis 37.6±16.0 years), mean LVEF and LVEDD at last follow-up was 45.5±6.6% and 58.5±3.6 mm, respectively (Table 2). One quarter of these patients (3/12) had non-truncating variants. Taking into account that only 4 subjects without DCM at first evaluation had a non-truncating variant (Table 1), 75% of them developed DCM, as compared with 21.4% in subjects with truncating variants (p=0.02) (Table 2). ECG features at first evaluation were comparable between subjects who developed or did not develop DCM (Table 2).

Regarding patients with DCM at first evaluation (n=74, 67.6% males, 40.1±13.2 years), no significant changes in LVEF (34.1±12.4% vs 34.1±13.0%; p=0.81) and LVEDD (64.0±9.3 vs 64.5±7.9 mm; p=0.66) were found after a median follow-up of 42.5 months (IQR: 6–94) (Table 3). Considering only DCM patients with available ECG at first evaluation and follow-up (n=53, 71.6%), 1 subject exhibited new atrial fibrillation

(1.8%), 8 subjects showed new negative T waves (17%) and mean QRS duration was 103.1±24.4 ms as compared with 98.6±21.5 ms at initial evaluation (p=0.10) (Table 3).

Clinical events

A total of 114 subjects were followed for a median of 38 months (IQR: 7–95). During this period, 9 patients died (7.9%; 77.8% cardiac deaths and 22.2% non-cardiac). Among the cardiac deaths, 2 were SCD (28.6%). Three additional patients presented an aborted SCD, while 4 patients received a LVAD and 17 underwent HTx (14.3%), including one of the patients who had an aborted SCD. Only 1 patient had an LVAD at last evaluation (Figure 1). The remaining cardiac deaths were due to HF (n=2) or to cardiac complications after HTx (n=3) (Figures 1 and 2).

Regarding arrhythmic events, SVT occurred in 3 patients and VF was documented in the 3 patients with aborted SCD (Figure 2). At last follow-up, 12 out of the 61 non-transplanted DCM patients with available follow-up data had an ICD implanted (19.7%), 91.7% for primary prevention. Only 1 patient had received an appropriate ICD shock.

Composite end-point of cardiac death, heart transplant, LVAD, aborted SCD or serious ventricular arrhythmia occurred in 24 of the patients with DCM (30.1%) (Figure 2), and the incidence of the composite end-point was of 5.1% per year. No events were found in individuals with *BAG3* mutation without DCM phenotype.

Predictors of adverse events

DCM patients (n=78) who reached the composite end-point of cardiac event (n=24, 83.3% males, 40.4±15.0 years at last follow-up) exhibited lower LVEF at first evaluation (24.1±9.9% vs 42.4±14.0%; p<0.001) and higher LVEDD (68.7±8.1 vs

60.9±8.5 mm; p=0.002) than DCM patients who did not have adverse events (Table 4). Non-sustained ventricular tachycardia on ECG Holter was more frequent in DCM patients with adverse events (57.1 vs 33.3%), but this difference did not reach statistical significance (p=0.26). Male sex was also more prevalent in patients who had events (Figure 3, Central Illustration). However, electrocardiographic features and the type of mutation did not differ between DCM patients with or without cardiac events (Table 4). The subgroup of patients with HF-related events (HTx, LVAD or HF death, n=20) were more frequently males and exhibited a lower LVEF and a larger LVEDD at first evaluation. No other clinical predictors were observed (Supplementary material table S2). Regarding the subgroup of DCM patients with serious arrhythmic events (n=8), male sex was not significantly associated with these complications but LVEF at first evaluation was still lower as compared with DCM patients without arrhythmic events (Supplementary material table S3).

Inmunohistochemistry

Immunofluorescence confocal microscopy of myocardial tissue from 3 patients with different truncating mutations in *BAG3* showed both myofibrillar disarray and a decrease and a relocation of BAG3 protein in the sarcomeric Z-disc, exhibiting a disorganized pattern (Figure 4 and Online Figures 1 and 2). By contrast, appropriate BAG3 localization was evident in the Z-disc region in DCM cases caused by *TTN* and *LMNA* mutations or without identifiable genetic cause (Figure 4).

Discussion

This study represents the largest cohort of DCM caused by *BAG3* mutations reported to date. Our findings show that DCM caused by mutations in *BAG3* is characterized by early onset disease in the majority of patients, with a high risk of progression to end-stage heart failure and a worse prognosis in men.

Furthermore, this is the first study to provide data on the impact of *BAG3* mutations on cardiomyocyte architecture in patients with DCM. Previous cardiac histologic data of *BAG3* mutations were limited to 2 patients with the p.Pro209Leu mutation, which causes restrictive cardiomyopathy and myofibrillary myopathy (19,20). In the present study, confocal microscopy of heart samples revealed that BAG3 protein was diminished and disorganized in the sarcomeric Z-disc in patients with truncating mutations in *BAG3* gene, which is similar to what has been observed in patients with the p.Pro209Leu mutation (20).

BAG3 protein interacts with the actin capping protein CapZ, stabilizing the myofibril structure in response to mechanical stress (21), and has been shown to be essential for the stability of the cardiac sarcomere (10). Accordingly, alteration in BAG3 location in the Z-disc could directly interfere with its function by preventing interaction with CapZ.

BAG3 is expressed mainly in skeletal muscle cells and cardiomyocytes, and mutations in the gene were initially reported to be associated with myofibrillar myopathy (16). Studies in knock-out mice showed striated muscle fiber degeneration and apoptotic nuclei, leading to fulminant skeletal myopathy and cardiomyopathy (9). However, to date, Pro209Leu is the only genetic variant that has been associated with a neuromuscular phenotype in humans (16). This mutation was not present in our cohort and there was no evidence of relevant neuromuscular involvement in any of our

patients. Only one patient with the p.Gln353Argfs*10 mutation and without a DCM phenotype had a single significantly increased creatine kinase value (1822 UI/L), from a sample taken soon after heavy training.

Natural history of BAG3 mutations

Our study provides detailed longitudinal data of a large cohort with *BAG3* mutations, allowing specific important information for physicians handling patients with this genetic diagnosis.

Our data show that 26.1% of genotype-positive individuals who were phenotype-negative at first evaluation developed LVEF impairment and LV dilatation during a median follow-up of 33 months. The mean age at DCM onset was 36.9 years and penetrance was 80% in subjects aged over 40 years (Central Illustration). Although this penetrance is high, it is lower than that of other genetic forms of DCM such as the recently described truncating filamin C form (*FLNC*), in which penetrance is almost complete over 40 years of age (22). Previous studies including DCM patients with *BAG3* mutations did not include detailed clinical information, but reported a mean age at DCM diagnosis of 44 and 37 years in the two largest cohorts reported to date from the US (22 patients) and Canada (21 patients), respectively (7,23).

Data on other factors that could have accelerated the onset of DCM phenotype, such as alcohol, pregnancy or infections (24,25), were unavailable in ours and previous cohorts. Nevertheless, the observed rate of progression into DCM phenotype is substantially higher than in other genetic DCM forms. For instance, *LMNA* genotype-positive phenotype-negative individuals were reported to develop left ventricular dilation in 24% of cases after a median follow-up of 7 years (26). Furthermore, the response to treatment in DCM patients with *BAG3* mutations seemed less prominent that in other

genetic forms of DCM, as only 2.9% of the patients with DCM at first evaluation normalized LVEF during follow-up (Figure 1). Although medical treatment data were not available in the present study, our results reveal a very low left ventricular reverse remodeling (LVRR) rate. In line with these results, a recent study has reported that rare genetic variants in genes that code for proteins of the cytoskeleton or Z-disc are independently associated with a lower rate of LVRR in DCM (27).

Prognosis of DCM caused by BAG3 mutations

Our study shows that patients with DCM caused by *BAG3* mutations have an adverse prognosis. The incidence of adverse cardiac events was 5.1% per year in patients with overt DCM phenotype (Figure 3). More men than women with DCM developed cardiac events during follow-up and the survival analysis showed a better prognosis in females. Male gender has also been found to be a predictor of worse outcome in other forms of genetic DCM such as those caused by mutations in *TTN* (28) or *LMNA* (26). However, male gender was not associated with serious arrhythmic events in our cohort. In fact, serious arrhythmias were infrequent with a rate of only 1% per year in all individuals with a *BAG3* mutation and 1.5% in those patients with DCM phenotype. This phenotype contrasts with other genetic causes of DCM with a heavy burden of arrhythmia, such as mutations in *LMNA* (29) or *FLNC* truncating variants (22) and is more similar to DCM caused by *TTN* truncating variants, in which arrhythmic events are less common (30).

By contrast, the number of patients who developed HF adverse events was high. Up to 27% of DCM patients required HTx, LVAD or suffered an HF-related death (4.3% per year), which depicts a more “heart failure” oriented phenotype in DCM caused by *BAG3* mutations. Along this line, Norton *et al* reported that 8 out of 17 patients with

DCM caused by *BAG3* mutations underwent HTx or presented HF-related death in a non-European cohort (7). Overall, these data support a phenotype dominated by HF-related complications irrespective of patients´ geographical origin.

Although data on the prevalence of BAG3 mutations at all participant centers was not available, the prevalence found at 2 of the participant centers (Hospital Puerta de Hierro in Madrid, Spain and The Cardinal Stefan Wyszyński Institute of Cardiology in Warsaw, Poland) was 6.7% (14/208), whereas it ranged from 2.3% to 3.6% in previous reports (6,7,13). Interestingly, the Hospital Puerta de Hierro and The Cardinal Stefan Wyszyński Institute of Cardiology are centers with important heart transplant/LVAD programs that could have enriched the BAG3 mutation uptake and potentially reflect again a "heart failure" oriented phenotype.

Other factors associated with adverse outcomes in our study were initial LVEF and LVEDD. However, there was a trend towards more arrhythmic events in subjects with a later DCM onset (p=0.1, Supplemental material Table S3). As late debuts were not very frequent, these results again underline the fact that prognosis is determined by an HF-related phenotype, especially in young patients.

In recent years, advances in pharmacological and non-pharmacological treatments have substantially improved the prognosis of DCM, and the estimated survival free from death or HTx in DCM have been reported to be 85% at 10 years (31). Thus, the 4.3% yearly rate of HF-related events found in our study highlights the aggressive course of *BAG3* DCM. This information and the factors associated with adverse events identified in this study could help physicians in referring *BAG3* DCM patients promptly for heart transplant evaluation and to promote aggressive pharmacological treatment from early stages of the disease.

Limitations

Follow-up data were not available in all the patients. The database was not designed to assess response to pharmacological treatments and data regarding this issue are lacking. Finally, the immunohistochemical analysis was only performed in heart tissue from individuals with truncating mutations and the effect of missense variants could not be evaluated.

Conclusions

DCM caused by mutations in *BAG3* is characterized by an aggressive clinical course dominated by heart failure complications and a high but incomplete penetrance in carriers over 40 years. Male gender, low LVEF and increased LVEDD at first evaluation were associated with an adverse prognosis during follow-up.

Perspectives:

Competency in medical knowledge**:**

Patients with dilated cardiomyopathy caused by *BAG3* mutations exhibit a high rate of heart failure-related events. Thus, aggressive and early treatment with heart failure drugs in DCM patients and a close follow-up in genotype-positive relatives is warranted.

Translational outlook:

As DCM penetrance is incomplete in *BAG3* mutations, further studies are needed to identify possible factors and mechanisms by which affected individuals develop DCM. Animal models could be useful to achieve this objective.

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Figures:

Central Illustration. Clinical picture and outcomes of 129 patients with *BAG3* mutations

SVT: Sustained ventricular tachycardia

Figure 1. Flowchart of patients included in the study.

DCM: Dilated cardiomyopathy, IQR: Interquartile range, HTx: Heart transplantation, LVAD: Left ventricular assist device, SCD: Sudden cardiac death

*:1 patient still on LVAD at last evaluation

Figure 2. Clinical events during follow-up of individuals with *BAG3* mutation

ICD: Implantable cardioverter defibrillator, SCD: Sudden cardiac death

*****: Sustained ventricular tachycardia and ventricular fibrillation.

Figure 3. Survival analysis in *BAG3* DCM.

A. Global survival free of the primary composite endpoint (heart transplantation, left ventricular assist device, cardiac death, aborted SCD and appropriate ICD shock). Analysis from date of first evaluation until last follow-up or event.

B. Male gender as risk factor for clinical events.

HF: Heart failure, ICD: Implantable cardioverter defibrillator, SCD: Sudden cardiac death

Figure 4. BAG3 localization in explanted hearts.

Samples fluorescently labeled for actinin (green) and BAG3 (red). Scale bar: 50 μm.

Left column: The first row belongs to a patient without mutations. The second to a patient with a mutation in Lamin, and the third and fourth rows to patients with truncating mutations in *TTN*. Samples labeled with actinin and BAG3 show normal localization of the proteins on Z-discs on all the patients.

Right column: The first row belongs to a patient without mutations. Subsequent rows belong to patients with *BAG3* truncating mutations. In patients with *BAG3* mutations, BAG3 is present at Z-discs but appears disorganized. Myofibrillar disarray can also be observed.

Tables

Table 1: Baseline characteristics of individuals with *BAG3* mutation.

INDEL: Insertion-deletion, LVEF: Left ventricular ejection fraction, LVEDD: Left ventricular end-diastolic diameter, NYHA: New York Heart Association, SD: Standard deviation, TAPSE: tricuspid annular plane systolic excursion

Table 2. Clinical characteristics of *BAG3* mutation genotype-positive relatives who develop DCM during follow-up compared with genotype-positive relatives who remain phenotype-negative

LVEF: Left ventricular ejection fraction, LVEDD: Left ventricular end-diastolic diameter, NYHA: New York Heart Association, SD: Standard deviation, TAPSE: tricuspid annular plane systolic excursion

[1]: 1 patient presented junctional rhythm; [2]: 1 patient presented atrial fibrillation.

Table 3. **ECG and echocardiographic characteristics of *BAG3* DCM**

LVEF: Left ventricular ejection fraction, LVEDD: Left ventricular end-diastolic diameter, NYHA: New York Heart Association, SD: Standard deviation, TAPSE: tricuspid annular plane systolic excursion.

*: 6 lost to follow-up and 2 patients with normalized phenotype (included). Last echocardiogram and ECG before heart transplantation in the 17 transplanted patients.

[1]: Only patients with available first and follow-up tests have been included.

Table 4. Clinical predictors of heart transplant, death, sudden cardiac death and serious arrhythmic events in individuals harboring *BAG3* mutation.

LVEF: Left ventricular ejection fraction, LVEDD: Left ventricular end-diastolic diameter, NYHA: New York Heart Association, SD: Standard deviation, TAPSE: tricuspid annular plane systolic excursion.

Table 1: Baseline characteristics of individuals with *BAG3* mutation

	Total cohort	**Phenotype-negative at first evaluation**	**DCM at first evaluation**
Number of subjects	129	55	74
Male (%)	80 (62)	30 (54.5)	50 (67.6)
Mean age at first evaluation ± SD	35.1±15.0	28.9±15.0	40.1±13.2
Type of mutation (%)			
• Truncating	111 (86)	51 (92.7)	60 (81.1)
• Non-truncating	18 (14)	4 (7.3)	14 (18.9)
Creatine kinase levels (UI/L) ± SD	129.3 ± 208.9	148.4 ± 323.7	109.5 ± 68.3
ECG			
Sinus rhythm (%)	118/121 (97.5)	52/52 (100)	66/69 (95.6)
QRS duration (ms)	94.7 ± 17.2	88.5 ± 10.7	99.4 ± 20.3
Negative T wave all locations (%)	21/109 (19.3%)	8/50 (16%)	13/59 (22%)
Echocardiogram			
LVEF % ± SD	44.4 ± 17.0	59.7 ± 5.7	32.9 ± 13.1
LVEDD (mm) ± SD	58.0 ± 9.8	50.3 ± 6.0	64.3 ± 7.7
TAPSE (mm) ± SD	21.6 ± 4.5	22.1 ± 3.1	19.1 ± 4.9

NYHA			
I	76/128 (59.3%)	52/54 (96.4%)	24/74 (32.4%)
II	20/128 (15.6%)	2/54 (3.6%)	18/74 (24.4%)
III	17/128 (13.3%)	0/54 (0.0%)	17/74 (22.9%)
IV	15/128 (11.7%)	0/54 (0.0%)	15/74 (20.3%)

LVEF: Left ventricular ejection fraction, LVEDD: Left ventricular end-diastolic diameter, NYHA: New York Heart Association, SD: Standard deviation,

TAPSE: tricuspid annular plane systolic excursion

Table 2. Clinical characteristics of initially unaffected *BAG3* genotype-positive relatives who developed DCM compared with those who did not develop DCM at last follow-up

	Normal phenotype after follow-up	**DCM after follow-up**	**p**
Number of subjects	34	12	
Male (%)	20/34 (58.8)	5/12 (41.7)	0.31
Mean age at first evaluation ± SD	26.2 ± 13.7	27.9 ± 11.5	0.70
Mean age at last evaluation ± SD	32.0 ± 13.6	37.3 ± 12.5	0.25
Median follow-up, months (IQR)	28 (5-67.3)	59 (25-190)	0.13
Creatine kinase levels (UI/L) ± SD	80.3 ± 26.4	105.7 ± 51.3	0.12
Non-truncating variants (%)	1/34 (2.9)	3/12 (25)	**0.02**
ECG			
Sinus rhythm first ECG (%)	33/33 (100)	10/10 (100)	0.58
Sinus rhythm follow-up ECG (%)	32/33 (97.0)[1]	11/11 (100)	0.70
QRS duration first ECG (ms)	88.6 ± 10.4	85.1 ± 15.3	0.56
QRS duration follow-up ECG (ms)	92.0 ± 11.7	105.8 ± 24.2	0.025
Negative T waves first ECG (%)	4/32 (12.5)	2/11 (18.2)	0.55
Negative T waves follow-up ECG (%)	4/32 (12.5)	2/10 (20.0)	0.76
Echocardiogram			
LVEF % first evaluation ± SD	59.9 ± 5.2	57.9 ± 4.4	0.26
LVEF % follow-up ± SD	56.6 ± 5.6	45.5 ± 6.6	<0.001

LVEDD (mm) first evaluation ± SD	49.5 ± 4.9	52.6 ± 8.7	0.30
LVEDD (mm) follow-up ± SD	50.1 ± 4.8	58.5 ± 3.6	<0.001
TAPSE (mm) first evaluation ± SD	21.4 ± 2.6	25.3 ± 1.9	**0.01**
TAPSE (mm) follow-up ± SD	22.3 ± 2.5	22.6 ± 5.0	0.87

LVEF: Left ventricular ejection fraction, LVEDD: Left ventricular end-diastolic diameter, NYHA: New York Heart Association, SD: Standard deviation, TAPSE: tricuspid annular plane systolic excursion.

[1]: 1 patient showed junctional rhythm

Table 3. ECG and echocardiographic characteristics of *BAG3* DCM

	First evaluation	Last evaluation*	p
Number of subjects	74	68	
Male (%)	67.6	66.1	
Mean age ± SD	40.1 ± 13.2	45.3 ± 13.5	
ECG			
Sinus rhythm (%)	51/53 (96.2)	50/53(94.3)	0.50
QRS duration (ms)	98.6 ± 21.5	103.1 ± 24.4	0.10
Negative T waves (%)	8/47 (17)	16/47 (34.0)	**0.04**
Echocardiogram			
LVEF % ± SD	34.1 ± 13.0	34.1 ± 12.4	0.99
LVEDD (mm) ± SD	64.5± 7.9	64.0 ± 9.3	0.66
TAPSE (mm) ± SD	19.1 ± 4.9	20.2 ± 5.3	0.25

LVEF: Left ventricular ejection fraction, LVEDD: Left ventricular end-diastolic diameter, NYHA: New York Heart Association, SD: Standard deviation, TAPSE: tricuspid annular plane systolic excursion.

*: 6 patients lost to follow-up and 2 patients with normalized phenotype (included). Last available echocardiogram and ECG before heart transplantation in the 17 transplanted patients.

Table 4. Clinical parameters in DCM patients with *BAG3* mutations classified according to the presence of cardiac events during follow-up

	DCM patients without cardiac events (n=54)	**DCM patients with cardiac events (n=24)**	**p**
Male sex (%)	55.6	83.3	**0.02**
Truncating mutation (%)	77.8	83.3	0.58
Non-truncating mutation (%)	22.2	16.7	0.58
Age at DCM onset	38.0 ± 12.3	33.4 ± 14.4	0.18
QRS width (ms) on 1st ECG	98.4 ± 21.8	96.7 ± 19.2	0.79
Negative T waves on 1st ECG (%)	17.0	33.3	0.18
LVEDD (mm) on 1st echo	60.9 ± 8.5	68.7 ± 8.1	**0.002**
LVEF (%) on 1st echo	42.4 ± 14.0	24.1± 9.9	**<0.001**
TAPSE (mm) on 1st echo	20.5 ± 5.1	15.5 ± 2.1	0.21
NSVT on Holter monitor (%)	33.3	57.1	0.26
CK (UI/L)	116.2 ± 67.9	89.7 ± 58.8	0.22

CK: Creatine kinase, LVEDD: Left ventricular end diastolic diameter, LVEF: Left ventricular ejection fraction, NSVT: non-sustained ventricular tachycardia, TAPSE: tricuspid annular plane systolic excursion.