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Case Report

Dilated cardiomyopathy due to a novel combination of TTN and BAG3 genetic variants: From acute heart failure to subclinical phenotypes

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ABSTRACT

Dilated cardiomyopathy (DCM) is defined as left ventricular enlargement accompanied by systolic dysfunction not explained by abnormal loading conditions or coronary heart disease. The DCM clinical *spectrum* is broad, ranging from subclinical to severe presentation with progression to end stage heart failure. To date, different genetic *loci* have been found to have moderate/definitive evidence for causality in DCM and pathogenic variants in the *TTN* gene represent the main genetic determinant.

Here, we describe a family in which the co-occurrence of two genetic hits, one in the *TTN* and one in the *BAG3* gene, was associated with heterogeneous clinical presentation ranging from subclinical phenotypes to acute cardiogenic shock mimicking fulminant myocarditis. We hypothesize that at least some specific *BAG3* genotypes could be related to DCM presenting with acute heart failure and suggest that patients and relatives carrying *BAG3* pathogenic variants should be addressed to a tertiary-level heart care center.

1. Introduction

Dilated cardiomyopathy (DCM) is a heart muscle disease defined by left or biventricular dilatation and systolic dysfunction in the absence of coronary artery disease, hypertension, valvular disease, or congenital heart disease [1]. Genetic variants play a pivotal role in the development of DCM. Hundreds of genes have been associated with an increased risk of developing DCM. However, to date, only 16 *loci* have been found to have moderate/definitive evidence for causality and at least one mutation is present in about 40% of familial DCM cases [2]. The clinical *spectrum* of genetically related DCM is broad, ranging from overt to subclinical presentation, highlighted by echocardiogram and cardiovascular magnetic resonance [3]. The penetrance of DCM-associated genetic alterations is indeed incomplete with up to 24% of mutated cases showing a subclinical form of the disease [3]. Nevertheless, the *spectrum* of pathogenic mutations in symptomatic and subclinical DCM has been shown to be similar [3].

Pathogenic variants in the *TTN* gene, encoding the giant muscle filament titin, are the main genetic cause of DCM [3]. Titin is critical for restoring the ability of the cardiac sarcomere to maintain optimal function of the left ventricle during diastole [4]. Titin also responds to mechanical stress by recruiting protein-binding partners involved in mechano-signaling, calcium signaling, cardiac metabolism, and protein quality control (PQC) [5]. PQC is an essential cellular function responsible for maintaining protein homeostasis, with a special significance in cardiac myocytes, as muscle contraction increases heat and tension favouring proteins unfolding or mutation [5]. In this context, chaperones play a central role in

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cellular maintenance by inhibiting aggregation of non-native proteins, and refolding misfolded proteins to their native state [5].

In 2011 Norton et al. reported that variants in the *BAG3* gene, a co-chaperone member of the Bcl2-associated athanogene (BAG) family, caused DCM [6]. BAG3 is essential for chaperones' activity and regulates numerous biological processes including stabilization of the sarcomere, activation of autophagy, and inhibition of apoptosis [6]. Under cellular stress, BAG3 acts with the chaperone Hsp70 to degrade misfolded proteins [7,8] inducing selective macroautophagy with uptake of protein aggregates by lysosomes [8].

TTN pathogenic alterations occur in ~18%-25% of DCM cases [9], while pathogenic variants of *BAG3* account for ~0.3% [10]. Since the penetrance of *TTN* alterations is incomplete [3,9], the occurrence of additional genetic hits has been proposed to modulate disease penetrance and phenotypic expression [9]. However, a recent population study highlighted that a second genetic alteration does not seem to influence the disease course of DCM both in patients and in mice [11], even if the identification of novel and different gene combinations might lead to different clinical outcomes.

Here, we describe a family in which the co-occurrence of two genetic hits, one in the *TTN* and one in the *BAG3* gene, led either to an early-onset DCM, presenting with acute cardiogenic shock mimicking fulminant myocarditis, or with subclinical phenotypes.

2. Case study

2.1. Clinical history and cardiac pathologic features

The proband was a 19-year-old male who presented to the emergency department with severe dyspnea that had increased over the past few days. About 2 weeks before he had had an abrupt onset of a flu-like syndrome with cough, asthenia, pallor, and intermittent fever. The patient reported having been in good health in the past and that he had practiced sports intensively. In addition, he denied alcohol abuse, drug use and cigarette smoking. He was admitted to the emergency room in cardiogenic shock (ejection fraction, EF:10%) with biventricular dysfunction, bilateral pleural effusion, and acute renal failure. C-reactive protein level was moderately elevated (9.69 mg/dL). Considering the ongoing hemodynamic instability, orotracheal intubation was carried out and a temporary cardiac assistor with an oxygenator was utilized. A few hours after the hospitalization, multiple biopsies from both ventricles performed during the procedure showed cardiomyopathic changes in the absence of histologic features of acute myocardial damage or inflammatory infiltrates (Fig. 1A). Immunohistochemical staining to highlight the presence of B (CD20) and T (CD3) lymphocytes and macrophages (CD68) gave negative results, according to the ESC criteria (Supplementary Figure 1) [12]. After a week from the onset of symptoms and without any signs of recovery and unsuccessful attempts to wean off assistance/drugs, an orthotopic cardiac transplant was successfully carried out. The patient was observed in January 2023, after the major pandemic COVID peaks, and resulted negative to the COVID test.

On gross examination, the explanted heart weighed 430 grams and showed marked biventricular dilation. Coronary arteries and cardiac valves were unremarkable, and there was no evidence of myocardial scarring. Histology confirmed the biopsy findings of myocyte hypertrophy and vacuolation, in absence of inflammatory infiltrates, necrosis, interstitial or replacement fibrosis (Figs. 1B-D). Polymerase chain reaction (PCR) and reverse transcriptase PCR analysis on myocardium did not detect any cardiotropic virus. The search for the most common bacterial and viral sources of infection, performed on serum and on a nasopharyngeal swab, gave negative results. High sensitivity cardiac troponin (NV<14 ng/L) and NT-ProBNP (NV<450pg/mL) were elevated in the patient being on admission 566 ng/L and 4,184 pg/mL, respectively.

Thereafter the heart transplantation, the proband was evaluated by a geneticist who ordered genetic tests and collected his family history. The mother had died at age 52 years for a clear cell renal cancer. His father was 56 years old, had previously experienced ventricular extrasystoles, which led to several cardiac investigations, including ergometric tests, without evidence of ischemia or stress-induced complex arrhythmias. Nevertheless, a past echocardiography had reported a mild systolic dysfunction, characterized by a reduction of the ejection fraction (EF:46%) due to mild segmental hypokinesia. Coronary ventricular angiography (CVG) had showed no significant coronary stenosis and cardiac magnetic resonance (CMR) had not revealed fibrosis or edema. In the following years, he no longer performed cardiological checks. The proband had also two asymptomatic siblings. His sister, who was 30 years old, was affected by moderate mental retardation. His brother, aged 26 years, had been practicing competitive sports for several years, with negative preparticipation cardiological screening.

2.2. Genetic analyses

Whole exome sequencing (WES) was performed on the proband's genomic DNA extracted from peripheral blood through Nextera Exome kit on the NextSeq2000 sequencer (Illumina, San Diego, CA, USA). Sequencing reads were aligned to the human reference genome (UCSC hg19). Variant calling was performed by GATK (v1.6-23-gf0210b3) for point variations and by eVai 3.1 (EnGenome) for Copy Number Variations (CNVs). The DNA variants were annotated by eVai and filtered by MAF<0.01 (GnomAD v2.1) and by the HP:0001638 (cardiomyopathy) phenotype as defined by the human phenotype ontology database. Filtered variants were classified according to ACMG/AMP criteria [13] and their familial segregation was tested by Sanger sequencing.

The proband was found to carry two nonsense variants: the *TTN*: NM_001267550.2:c.96697C>T (p.Arg32233Ter) and the *BAG3:* NM_004281.4:c.361C>T (p.Arg121Ter). Both alterations were identified in heterozygosity, and were predicted to induce nonsense-mediated mRNA decay by the AutoPVS1 software (https://autopvs1.bgi.com/). The *TTN*:c.96697C>T maps in the A-band region of the N2-B isoform of the titin protein and is constitutively expressed in *TTN* transcripts (percent spliced-in (PSI) of exon 174 = 100%). Both variants are rare based on population cohorts in the Genome Aggregation Database (MAF in gnomAD 2.1 = 0.0004789% for *TTN*:c.96697C>T; MAF in gnomAD 2.1 = 0% for *BAG3*:c.361C>T). The *TTN*:c.96697C>T has already been reported in a patient with hypertrophic cardiomyopathy [14] and the *BAG3*:c.361C>T has already been reported in patients with DCM [15–18]. According to ACMG/AMP_2015 guidelines, both variants were classified as pathogenic by the following criteria: PVS1_very strong, PM2_supporting and PS4_moderate.

By familial segregation analysis, both the *TTN*:c.96697C>T and the *BAG3*:c.361C>T resulted to be paternally inherited and both present, in heterozygosity, in the brother. The unaffected sister did not carry any of the two variants (Fig. 2A).

2.3. Cardiological and clinical assessment of the family

Based on the genetic results, both the proband's father and brother underwent a novel cardiological assessment. An electrocardiogram and 24-Holter monitoring, as well as complete transthoracic echocardiography were performed with a 2D Global systolic Longitudinal myocardial Strain (GLS) using speckle-tracking method [19]. In addition, a CMR for tissue characterisation with T2-weighted sequences and Late Gadolinium Enhancement (LGE) was performed.

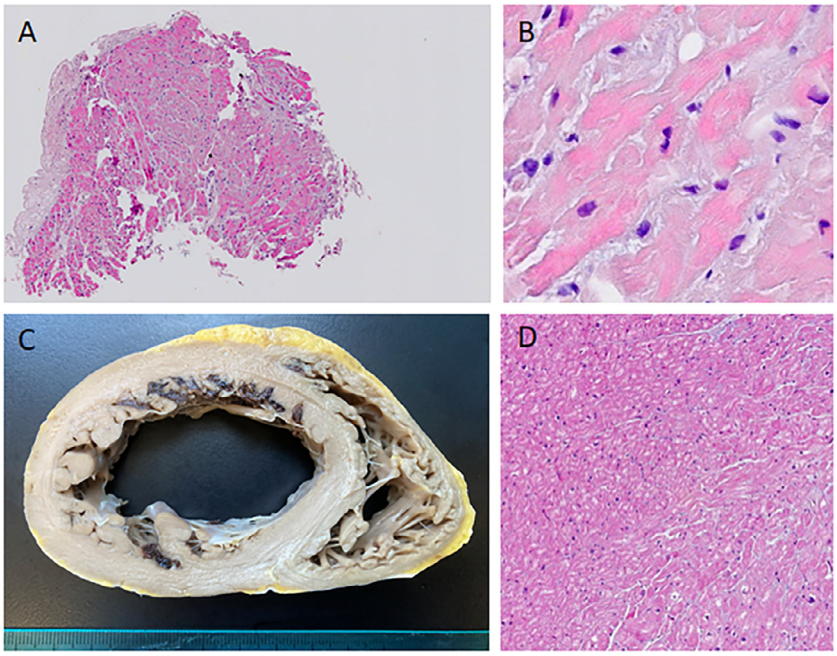

Fig. 1. Heart morphology of the proband. (A,B) Endomyocardial biopsy showing cardiomyopathic features consisting of cardiac myocyte hypertrophy and interstitial fibrosis in the absence of inflammatory infiltrates and acute cardiac myocyte damage. (C) Short-axis section of the explanted heart showing biventricular eccentric hypertrophy, with prominent dilation of both cavities. In the left ventricular cavity, trabecular recesses are filled with thrombotic material. (D) Histological section from the explanted heart showing cardiac myocytes hypertrophy and vacuolation in the absence of inflammatory infiltrates (Hematoxylin and eosin, original magnification 20X).

The father was found asymptomatic with no signs of congestive heart failure (NYHA functional class I). The electrocardiogram (EKG) showed an abnormal repolarization with anterolateral negative T waves. The echocardiogram (ECG) evidenced a mild ventricular dilation and systolic dysfunction characterized by a mild reduction of EF (47%) along with a low GLS value (-13%) due to hypokinesis of the lower septum and middle basal inferolateral wall. These findings were confirmed at CMR which also showed the presence of small areas of intramural and subepicardial laminar LGE in inferoseptal and inferolateral area.

The proband's brother was assessed as a NYHA I functional class. The EKG showed a sinus rhythm (cardiac frequency: 80bpm). The ECG identified normal value of EF (50%). Interestingly we found an abnormal value of GLS (-15%) corresponding to the segmental subepicardial and midwall inferolateral LGE areas discovered by CMR, that confirmed the EF value and displayed a subepicardial and midwall inferolateral LGE.

Fig. 2B shows the CMR, transthoracic ECG and EKG images from the proband, his father and his brother, highlighting the variability of the penetrance of the *TTN-BAG3* double hit.

Since some *BAG3* variants have been associated to progressive myofibrillar myopathy with markedly increased serum creatine phosphokinase (sCPK) level [20], sCPK was measured in the proband as well as in his father and brother. However, the sCPK level was within the normal range for all three siblings.

2.4. Titin and BAG3 myocardial expression analyses

To assess whether the described genetic alterations interfered with the myocardial expression of the two proteins, we performed immunohistochemistry and western blot (WB) analysis with polyclonal antibodies against titin (PA5-52379, Invitrogen) and BAG3 (AB47124, Abcam) on tissue sections from index cases and control hearts: 1) donor heart not implanted for technical reasons (n=3); 2) recipient heart with idiopathic dilated cardiomyopathy (n=1) and 3) recipient heart with ischemic heart disease (n=1). Based on immunohistochemical staining, both proteins were expressed in the myocardium of the index case, although a less defined cytoplasmic striation with diffuse granularity was observed in the affected heart compared to the controls (Fig. 3A). According to WB analysis, a slight reduction in BAG3 expression in the proband compared to controls was evident when proteins were normalized for actin (Fig. 3B). No aberrant bands consistent with truncated BAG3 protein expression were observed in several replicated experiments (n=5). Overall, our results point to haploinsufficiency as a pathogenic mechanism for the *BAG3*:c.361C>T (p.Arg121Ter) variant. Regarding titin expression, we performed analyses on silver-stained vertical SDS-agarose gels [21], but were unable to resolve the various titin isoforms clearly (not shown), thus the setup of additional experiments would be needed to address the issue of its pathogenetic mechanism.

3. Discussion

We describe a family with the co-occurrence of two genetic alterations: a truncating variant in *TTN* and a truncating variant in *BAG3*. Both genetic variants had already been independently associated with cardiomyopathy [15–18]. In our family, the disease phenotypic expression was very variable: the proband presented with acute heart failure leading to heart transplant at age 19, while his brother and father showed subclinical myocardial dysfunction at age 26 and chronic disease at age 56.

Heterozygous titin truncating variants (TTNtv) are known to be the most common genetic cause of familial dilated cardiomyopathy [22]. *BAG3* is another gene that has been definitively associated with autosomal dominant DCM (ClinGen database, https://search.clinicalgenome.org/kb/genes/HGNC:939;[6]). In 2018, Domínguez F et al. showed that DCM caused by mutations in *BAG3* is characterized by early-onset disease in many patients, with a high risk of progression to end stage heart failure in men [23].

BAG3 alterations have been also proposed as genetic modifiers of DCM course. Valerie D. Myers et al. reported that four common non-truncating *BAG3* alleles were not causative of disease but were

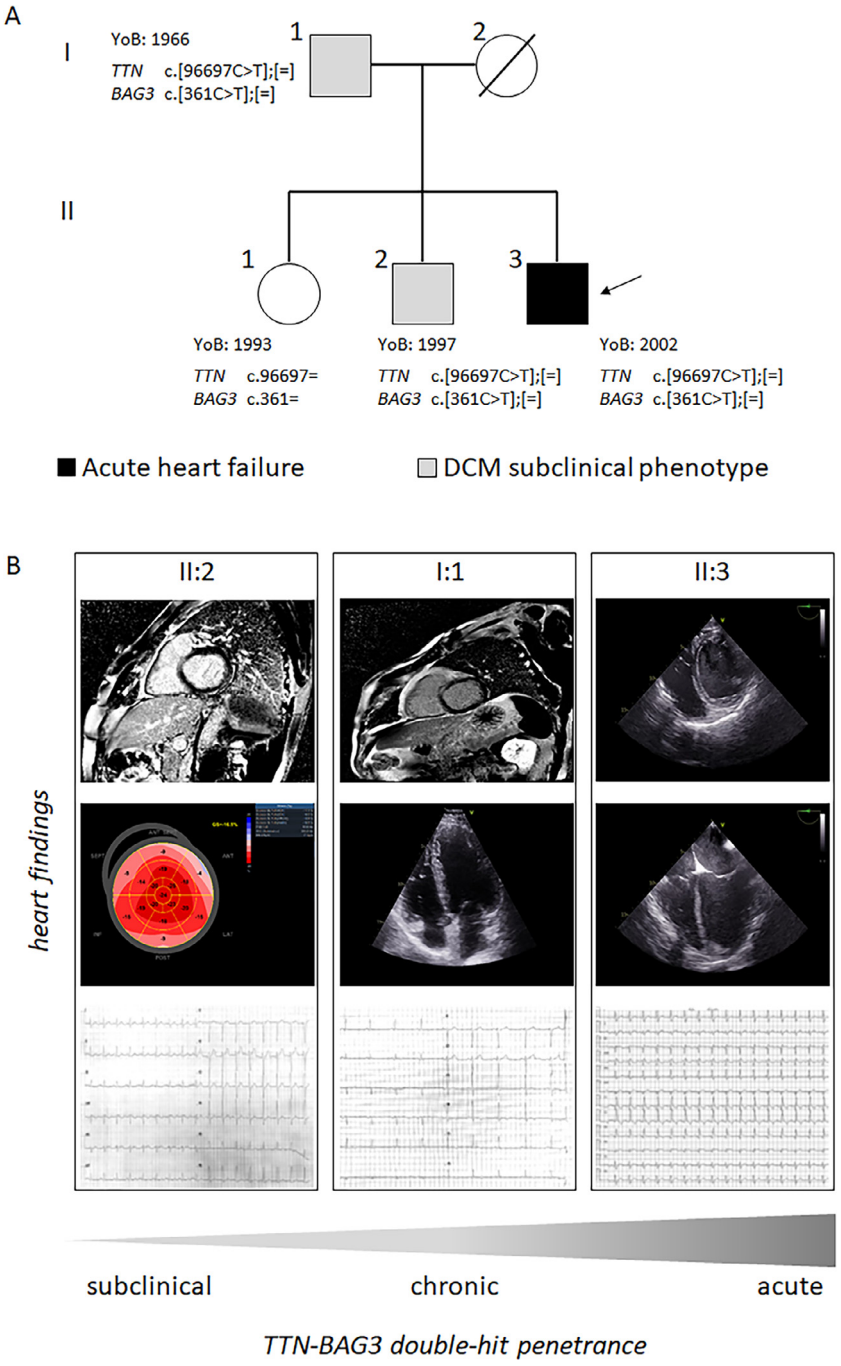

Fig. 2. Clinical and genetic features of the family with heterozygous truncating *TTN* and *BAG3* variants. (A) Family pedigree. The phenotype of each patient is shown with different filling motifs and the clinical legend is given under the pedigree. The *TTN* and *BAG3* genotypes are given under each case's symbol. YoB: year of birth. (B) Cardiac features of the proband, his father and his brother. The different penetrance (i.e. subclinical, chronic, acute) of the double-hit in *TTN* and *BAG3* is illustrated by the cardiac magnetic resonance, transthoracic echocardiogram and electrocardiogram images.

associated with a negative outcome in patients with DCM [24]. Interestingly, the four alleles were annotated in ClinVar database as benign, likely benign, or as variant of uncertain significance. To date, 108 pathogenic or likely pathogenic (P/LP) *BAG3* variants have been submitted to ClinVar database. Of those, ~91% are truncating while only ~5% are missense. Moreover, all the *BAG3* truncating variants present in ClinVar have been classified as P/LP, except those mapping in the last exon. On the other hand, the 96% of the all *BAG3* missense changes have been reported as variants of unknown significance. These data and the findings of Valerie D. Myers et al., 2018 [24], indicate that at least some of the missense variants could act as hypomorphic alleles modulating the disease outcome.

The molecular mechanisms underlying both *TTN* and *BAG3*-related DCM are not fully elucidated. Both haploinsufficiency and the "poison peptide" hypotheses have been proposed to explain the pathogenicity of TTNtv [25]. Also, at least some *BAG3* variants, even truncating, are expected to increase the protein expression [26]. In the present case, WB results, in line with AutoPVS1 tool prediction, point to haploinsufficiency as the possible pathogenic mechanism of the *BAG3* variant. More sophisticated techniques are needed to demonstrate the molecular mechanism associated with the TTNtv

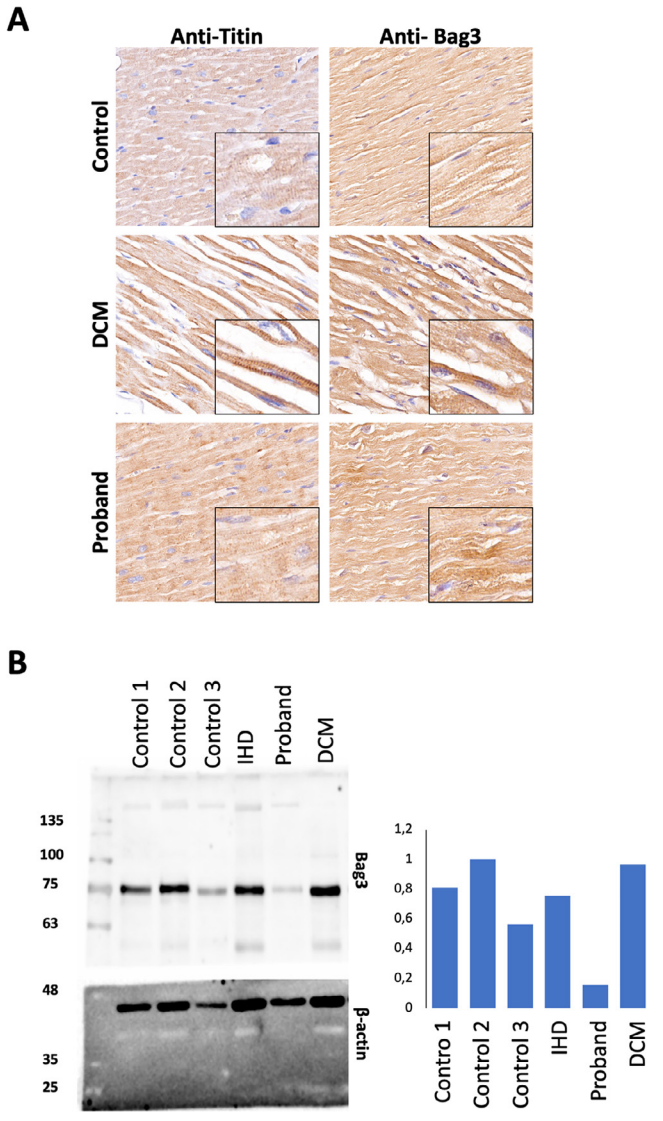

Fig. 3. Titin and BAG3 myocardial expression analyses. (A) Immunoistochemical analyses of TTN and BAG3 in formalin-fixed paraffin-embedded myocardial sections from the proband and controls (donor heart and idiopathic dilated cardiomyopathy, DCM). Original magnification 40X. (B) Representative western blot and densitometry of BAG3. Normalization relative to β-actin showed a slight reduction in the proband as compared to three donor hearts (controls), and two recipient hearts (ischemic heart disease, IHD and idiopathic dilated cardiomyopathy, DCM).

and the combined effects of the two genetic changes [25]. Very recently, Cabrera-Romero et al, showed that the penetrance of a new DCM diagnosis in genotype-positive relatives is ~11% over a median follow-up of 3 years, and that it is associated with different genetic and nongenetic factors [27]. However, the penetrance of concurrent genetic hits is still unknown, and studies focusing on multiple protein alterations are needed.

Alongside with genetic modifiers, also nongenetic hits, as infectious conditions, have been proposed to modulate the DCM expression [28]. A disease presentation like our proband has been previously described in four individuals carrying a monoallelic *BAG3* truncating variant [29]. In these patients, DCM manifested with acute heart failure shortly after an apparent viral infection. Three of them required emergent heart transplantation. The onset of the disease appeared at very young ages in two of the described patients (15 and 17 years, respectively). Diagnostic suspicion was of fulminant myocarditis. Screening for viral genomes was negative, however, as in our case, it cannot be excluded that this result was affected by the time lapse between the onset of symptoms and the analysis. Therefore, we believe that endomyocardial biopsy should be promptly performed in patients admitted to the intensive care unit for cardiogenic shock, to increase the probability to highlight a myocardial inflammation and/or the presence of viral genomes within the myocardium. On the other hand, based on the current findings and some evidence from the literature [22,28–30], we hypothesize that *BAG3* genotypes *per se* may be related to an acute presentation mimicking fulminant myocarditis, irrespective of patient age and occurrence of non-genetic hits [this report, [28]]. This hypothesis is supported by studies on animal models, as BAG3-deficient mice develop a fulminant myopathy characterised histologically by non-inflammatory myofibrillar degeneration with apoptotic features [31]. Furthermore, Wang J et al. demonstrated that decreased Bag3 levels lead to increased cardiac inflammasome [32]. Finally, recent evidence has shown that recessive alterations in another BAG protein (i.e. BAG5) cause DCM with advanced heart failure at a young age [33].

4. Conclusions

In patients presenting with cardiogenic shock without evidence of myocardial inflammation and/or viral infection, genetic analysis may reveal a causal role of unexpected genetic variance. Early endomyocardial biopsy proves crucial in this context. Patients carrying *BAG3* pathogenic variants should be addressed to a tertiary-level heart care center for the definition of the disease's course and for the management of relevant acute events.

Declaration of competing interest

All authors disclose any actual or potential conflict of interest.

CRediT authorship contribution statement

Irene Bottillo: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Carla Giordano:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. **Maria Pia Ciccone:** Formal analysis, Investigation, Methodology, Writing – original draft. **Maria Gemma Pignataro:** Data curation, Formal analysis, Investigation. **Fiammetta Albi:** Data curation, Formal analysis, Investigation. **Gabriella Parisi:** Data curation, Formal analysis, Investigation. **Daniela Formicola:** Data curation, Formal analysis, Methodology. **Simona Grotta:** Formal analysis, Methodology. **Federico Ranocchi:** Investigation, Methodology. **Maria Valeria Giuli:** Data curation, Formal analysis, Methodology. **Saula Checquolo:** Methodology, Formal analysis, Data curation. **Laura Masuelli:** Data curation, Formal analysis, Investigation. **Federica Re:** Data curation, Formal analysis, Investigation. **Silvia Majore:** Data curation, Investigation, Writing – review & editing. **Giulia d'Amati:** Data curation, Supervision, Validation, Writing – review & editing. **Paola Grammatico:** Funding acquisition, Resources, Supervision.

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Disclosures

None.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.carpath.2024.107675.

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