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CASE REPORT

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Double missense mutations in cardiac myosin-binding protein C and myopalladin genes: A case report with diffuse coronary disease, complete atrioventricular block, and progression to dilated cardiomyopathy

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Abstract

Cardiomyopathies caused by double gene mutations are rare but conferred a remarkably increased risk of end-stage progression, arrhythmias, and poor outcome. Compound genetic mutations leading to complex phenotype in the setting of cardiomyopathies represent an important challenge in clinical practice, and genetic tests allow risk stratification and personalized clinical management of patients. We report a case of a 50-year-old woman with congestive heart failure characterized by dilated cardiomyopathy, diffuse coronary disease, complete atrioventricular block, and missense mutations in cardiac myosin-binding protein C (*MYBPC3*) and myopalladin (*MYPN*). We discuss the plausible role of genetic profile in phenotype determination.

KEYWORDS
cardiac myosin-binding protein C, complete atrioventricular block, diffuse coronary atherosclerosis, dilated cardiomyopathy, myopalladin

1 | INTRODUCTION

Cardiomyopathies assessment represents a key role in the setting of heart failure and sudden death approach (McKenna, Maron, & Thiene, 2017). Compound genetic mutations leading to complex phenotype in the setting of cardiomyopathies represent an important challenge in clinical practice, and genetic tests are helpful in the clinical management of patients as they can provide important information on risk stratification, to achieve personalized therapy indicating differential surveillance strategies among individuals. Here, we report a case of a 50-year-old woman with congestive heart failure characterized by dilated cardiomyopathy, diffuse coronary disease and complete atrioventricular block, and genetic evidence of missense mutations in cardiac myosin-binding protein C (*MYBPC3*) and myopalladin (*MYPN*).

2 | CASE REPORT

A 50-year-old woman was referred to our cardiovascular department for ankles swelling and shortness of breath. An electrocardiogram showed sinus tachycardia and complete atrioventricular block with ventricular rhythm 40 bpm and diffuse ST depression (Figure 1). Then, the patient was admitted to the cardiology intensive care unit after a clear evidence of heart failure with peripheral congestion; she had a history of nontoxic multinodular goiter and no previous

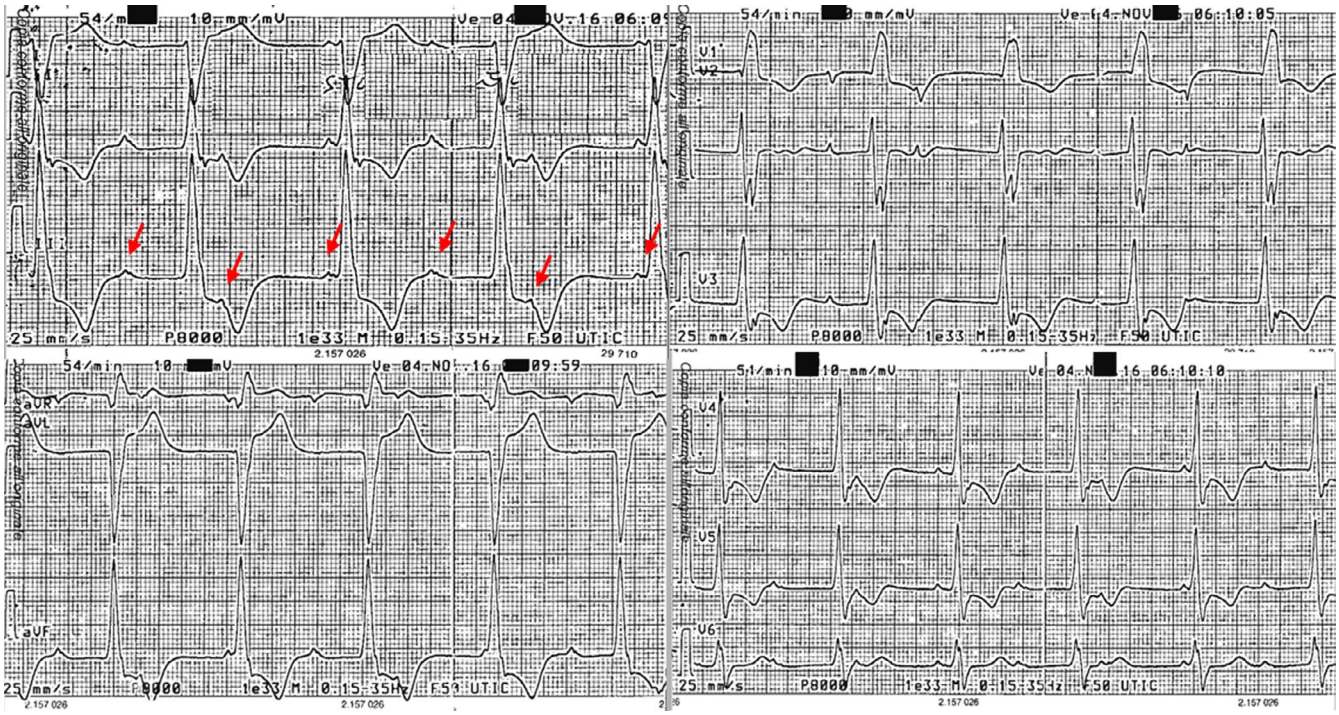

FIGURE 1 Sinus tachycardia and complete atrioventricular block with ventricular rhythm 40 bpm and marked diffuse ST depression (red arrows indicate P wave)

history of cardiovascular disease or cardiovascular risk factors. An echocardiogram demonstrated a dilated cardiomyopathy with end-diastolic diameter of the left ventricle (LV) of 62 mm, severely depressed left ventricle ejection fraction (LVEF), 20%, mild hypertrophic septum (13.5 mm), trabecular aspect of lateral and inferior LV wall, thrombus in the apex, and severe tricuspid regurgitation with inferior vena cava reflow (Figure 2). Furthermore, at a cardiac magnetic resonance, the short-term inversion recovery (STIR) detected subendocardial edema in the septum, anterior and lateral walls. Late sequences showed subendocardial necrosis at the level of the anterior-middle and later-middle segments, and full-thickness necrosis in the lateral-apical segment.

A coronary angiography showed calcified critical stenosis of many proximal vessels and diffuse peripheral atheromasic disease with consequent recommendation to surgical revascularization. As the patient was asymptomatic for angina with negative troponin test and in presence of the complete atrioventricular (AV) block, severe LV dysfunction and apical thrombosis, he underwent cardiac resynchronization therapy defibrillator (CRT-D) and started therapy with ACE-inhibitor, mineralocorticoid receptor antagonist, beta-blocker, warfarin, acetylsalicylic acid, and furosemide. After 1 month, we observed complete regression of apical thrombosis associated to clinical improvement with increased LVEF. Then, the patient underwent surgical myocardial revascularization with the mammary artery to anterior interventricular artery and venous by-pass to the obtuse marginal and posterior interventricular artery. Five months later, the patient was in sinus rhythm with ventricular CRT-D stimulation, NHYA class I, improved contractile function (LVEF 45%–48%) and mild tricuspidal insufficiency, still stable at 1 year follow-up.

Genetic analysis by next-generation sequencing (NGS), after informed consent of the patient, was performed for suspected genetically dilated cardiomyopathy and identified a double mutation in 11p11.2, cardiac myosin-binding protein C (*MYBPC3*) gene, and in 10q21.3, myopalladin gene (*MYPN*). We verified that this patient was heterozygous for two missense mutations: the first in gene *MYBPC3*: c.2459G > A, p.(Arg820Gln) in exon 24, and the second in gene *MYPN*: c.3335C > T, p.(Pro1112Leu) in exon 18.

3 | MATERIALS AND METHODS

3.1 | Libraries preparation and Next-generation sequencing (NGS)

Peripheral blood samples were taken from the patient, and genomic DNA was isolated by using Bio Robot EZ1 (Quiagen). The quality of DNA was tested on 1% electrophorese agarose gel, and the concentrations were quantified with Nanodrop 2000 C spectrophotometer (Thermo Fisher Scientific).

A library of all coding regions of the 76 genes, including genes related to hypertrophic cardiomyopathy, dilated cardiomyopathy, arrhythmogenic cardiomyopathy, and channelopathies, was obtained using the Haloplex target enrichment kit (Agilent Technologies) according to the manufacturer's instructions. The libraries were pooled, and NGS was performed on a MiSeq sequencer (Illumina) using a MiSeq Reagent kit V3 300 cycles flow cell. All putatively pathogenic variants were confirmed by Sanger sequencing. Polymerase chain reaction (PCR) products were sequenced using ABI Prism 3,100

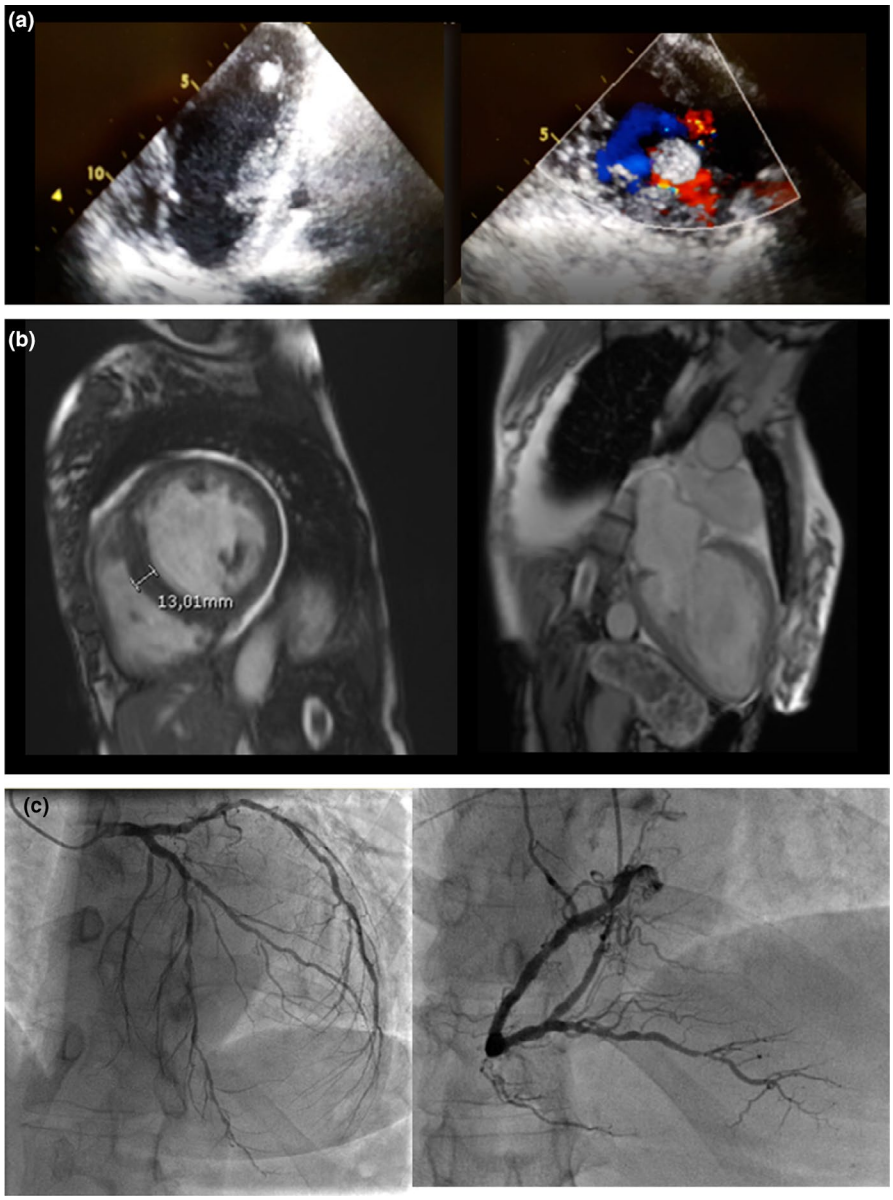

FIGURE 2 (a) Echocardiography evidence of left ventricular apical thrombosis and hypertrabeculation; (b) cardiac RMN showing septum hypertrophy and necrosis in the lateral-apical segment; (c) coronary angiography showing diffuse atherosclerosis

Genetic Analyzer (Thermo Fisher Scientifi) and the BigDye Terminator v1.1 sequencing kit (Applied Biosystems, Foster City, CA, USA).The produced raw paired-end reads underwent quality checking by using the FastQC tool [Andrews S. (2010). FastQC: a quality control tool for high throughput sequence data. Available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc] and then aligned to the hg19 reference genome sequence by means of Bowtie (Langmead & Salzberg, 2012). Depth of coverage statistics for the target regions was calculated by TEQC ver. 3.47 (Hummel, Bonnin, Lowy, & Roma, 2011). Variants were called by means of the HaplotypeCaller tool of GATK ver. 3.58 (McKenna et al., 2010), while functional annotation was carried out by ANNOVAR tool, using RefSeq gene and transcript annotations (updated to December 2016) (Wang, Li, & Hakonarson, 2010). Variants were found in dbSNP ver. 150 (Sherry et al., 2001), ExAC ver. 0.311 (Lek et al., 2016), and Exome Variant Server (http://evs.gs.washington.edu/EVS, accessed at December 2016), HRC (McCarthy, 2017), Kaviar (Glusman, Caballero, Mauldin, Hood, & Roach, 2011) and ClinVar (Landrum et al., 2017). Predictions of functional consequences for missense variants were further collected by querying the dbNSFP ver. 3.2 resource and retrieving precomputed pathogenicity predictions and evolutionary conservation measures (Liu, Jian, & Boerwinkle, 2011).

Different filtering strategies were implemented in order to determine the candidate causative variants for the patient. Interesting variants were those exhibiting relevant functional annotations (e.g., they were either missense, or splicing, or stopgain, or stoploss or frameshift mutations), clinical significance according ClinVar, low (≤0.05) or absent Minor Allele Frequency in public population databases, absence in healthy samples or presence in samples with same clinical traits.

4 | RESULTS

Next-generation sequencing revealed two missense variant in heterozygous state, one in the exon 17 of the *MYPN* gene (NM_032578) (OMIM 608,517) c.866G > C resulting in a p.(Pro1112Leu) substitution (GRCh37/hg19), and one in the exon 24 of the *MYBPC3* gene (NM_000256) (OMIM 600,958) c.2459 G > A p.(Arg820Glu). Variants were detected with a depth of coverage >706× and 662×, respectively, with elevate quality scores (i.e., Phred quality > 3,000 and genotype quality = 99). The amino acid substitution *MYBPC3* (Arg820Glu) was known (dbSNP ID: rs2856655) but very rare (0,00,002 estimated frequency by the ExAC and gnomAD database) and predicted as deleterious or probably damaging by several software tools, including SIFT, PolyPhen2, MutationTaster, CADD, and M-CAP, and is reported to be likely pathogenic in ClinVAr. The amino acid substitution *MYPN* p.(Pro1112Leu) was less rare (dbSNP ID: rs71534278; 0,003 estimated frequency by the ExAC and gnomAD database), and it is predicted as deleterious or probably damaging by several software tools, including SIFT, PolyPhen2, CADD, and M-CAP, but its contribution to the phenotype is not clear. The variants were confirmed by Sanger sequencing.

5 | DISCUSSION

In this case report, the coexistence of advance heart failure, atrioventricular block, significative and diffuse coronary atherosclerosis, and dilated cardiomyopathy in a relatively young woman without apparent cardiovascular risk factors led us to perform genetic screening, and believing the particular clinical picture could have a deeper pathological explanation.

In literature, the *MYBPC3* gene is responsible for 40%–50% of all hypertrophic cardiomyopathies (HCM) (Sabater-Molina, Pérez-Sánchez, Hernández Del Rincón, & Gimeno, 2018), but *MYBPC3* mutations can be associated to overlapping phenotype leading several forms of cardiomyopathies, as dilated cardiomyopathy (DCM) and left ventricular noncompaction (Sedaghat-Hamedani et al., 2017; Zhao et al., 2015). Most *MYBPC3* mutations are heterozygous and carriers often have a late disease onset with a benign disease progression (Carrier, Mearini, Stathopoulou, & Cuello, 2015). Konno and collaborators confirmed that elderly patients with the p.(Arg820Gln) mutation in the *MYBPC3* gene may show "burnt-out" phase HCM, with DCM phenotype appearance, characterized by LV systolic dysfunction with a diffuse LV hypokinesis, slow LV ejection fraction, and mild fibrosis, without myocardial hypertrophy and myofibrillar disarray at myocardial biopsy (Konno et al., 2003). This underlines the difficult differential diagnosis between "burnt-out" dilated HCM and DCM, as in our case, with a mild hypertrophic form evolved into dilatation.

The occurrence of complete atrioventricular block could have many explanations; among them, we could identify an ischemic-related block at infrahissian level, explained by diffuse coronary atherosclerosis. On the other hand, *MYBPC3* could be implicated by unknown mechanism, as already suggested by a previous case, a 16-year-old girl affected by missense mutation, with syncope and ICD implantation, and subsequent evidence of complete heart block (Walsh et al., 2012); furthermore, Chida and collaborators suggests a predictive role of *MYBPC3* mutation for sudden death in patient affected by hypertrophic cardiomyopathy, maybe related to abnormal heart conduction over ventricular arrhythmias (Chida et al., 2017).

Myopalladin mutations cause various forms of cardiomyopathy via different protein–protein interactions as disturbed myofibrillogenesis and nuclear shuttling and lead to abnormal assembly of terminal Z-disk within the cardiac transitional junction and intercalated disk (Purevjav et al., 2012). The p.(Pro1112Leu) residue of *MYPN* is well conserved among the orthologous proteins, the replacement of the polar proline for a hydrophobic leucine may not be pathogenic on its own, (Duboscq-Bidot et al., 2008), but the coexistence of other mutations may modulate the phenotype (Ingles et al., 2005; Tsoutsman, Bagnall, & Semsarian, 2008).

Myopalladin is part of the family members of palladin that includes also myotilin, expressed in skeletal and cardiac muscle.

The ability to connect with several molecules, in particular with α-actinin, localized along stress fibers and at the Z line of cardiac muscles, suggests that palladin family has the potential to serve as a cytoskeleton scaffold and signaling mediator (Jin et al., 2010). Palladin may also play a role in the pathology of myocardial infarction; several lines of evidence suggest that palladin is involved in vascular smooth muscle cell phenotypic switching and migration, contributing to formation and repair of lesion in response to vascular injury or atherosclerosis disease (Shiffman et al., 2005). In our patient suffering from diffuse coronary artery disease without the common cardiovascular risk factors, we hypothesized that the myopalladin mutation could favor the atherosclerosis process due to its similarity with the palladin, a critical paralog implicated in vascular plaque progression (Modos et al., 2016; Waard, Achterberg, Beauchamp, Pannekoek, & Vries, 2003). Whether confirmed in future studies, we would much more take in consideration genetic profiling in cardiac ischemic disease, ensuing strict cardiovascular risk assessment in patient offspring.

6 | CONCLUSION

Atrioventricular block can induce chronic heart failure, and electrocardiogram represents the gold standard for detection of rhythm disturbance. Furthermore, a complete evaluation of the patient, from anamnesis to cardiac resonance and coronary angiography, can lead to a better definition of clinical puzzle, while genetic profile allows the identification of genetic variants possibly implicated in the clinical picture. The missense mutations in *MYBPC3* gene (c.2459G > A, p.(Arg820Gln)) could be responsible for dilated cardiomyopathy and AV block, while the mutations in *MYPN* (c.3335C > T, p.(Pro1112Leu)) could be for the first time described in human as associated to diffuse and aggressive coronary atherosclerosis, and may contribute to phenotypic variability.

Cardiomyopathies caused by double-gene mutations are rare but conferred a remarkably increased risk of end-stage progression,

arrhythmias, and poor outcome. Compound genetic mutations leading to overlapping phenotype in the setting of cardiomyopathies represent an important challenge in clinical practice, and genetic tests are helpful in the clinical management of patients as they can provide important information on risk stratification, to achieve personalized therapy indicating differential surveillance strategies among individuals.

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CONFLICT OF INTEREST

Authors do not have conflict of interest.

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