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POST-MORTEM diagnosis of left dominant arrhythmogenic cardiomyopathy: THE IMPORTANCE of a multidisciplinary network for sudden death victims. “HIC mors gaudet succurrere vitae”

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PII: S1054-8807(19)30322-9

DOI: <https://doi.org/10.1016/j.carpath.2019.107157>

Reference: CVP 107157

To appear in: *Cardiovascular Pathology*

Received Date: 22 July 2019

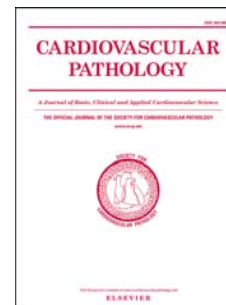
Revised Date: 17 September 2019

Accepted Date: 27 September 2019

Please cite this article as: Maddalena G, Ornella L, Alberto F, Valentina A, Raffaello D, Marco F, Cesare R, Luigi L, Marco S, Claudio R, POST-MORTEM diagnosis of left dominant arrhythmogenic cardiomyopathy: THE IMPORTANCE of a multidisciplinary network for sudden death victims. “HIC mors gaudet succurrere vitae”, *Cardiovascular Pathology*, <https://doi.org/10.1016/j.carpath.2019.107157>.

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POST-MORTEM DIAGNOSIS OF LEFT DOMINANT ARRHYTHMOGENIC
CARDIOMYOPATHY: THE IMPORTANCE OF A MULTIDISCIPLINARY NETWORK
FOR SUDDEN DEATH VICTIMS. “HIC MORS GAUDET SUCCURERE VITAE”.

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Abstract

An apparently healthy man died suddenly at the age of 49 during physical activity. The heart was referred to our Cardiovascular Pathology Unit for valve tissue banking. Pathology findings led to the diagnosis of arrhythmogenic left ventricular cardiomyopathy (ALVC). Molecular autopsy was performed and two variants of interest were identified in genes associated with arrhythmogenic cardiomyopathy. The 19-year-old son underwent a cardiac screening comprehensive of ECG, echocardiogram, cardiac magnetic resonance and genetic testing, and the diagnosis of ALVC was achieved.

This case report highlights the need of a systematic evaluation of all sudden death victims with autopsy performed by expert cardiovascular pathologists and implemented by molecular analysis, aiming to identify also rare hereditary diseases and activate proper family screening.

Key words: sudden death, cardiovascular pathology, molecular autopsy, multidisciplinary network, arrhythmogenic left ventricular cardiomyopathy, familial screening.

Introduction

Accurate autopsy examination in cases of sudden death (SD) is essential to identify the underlying disease and screen relatives. However, forensic autopsy may be inconclusive especially in cases of mild and/or uncommon abnormalities. A multidisciplinary approach specifically dedicated to SD victims increases the diagnostic yield as the cooperation between expert cardiovascular pathologists and clinicians may identify the cause of death. Moreover, an integrated post-mortem examination combining autopsy with molecular biology testing (molecular autopsies) has been proposed to identify genetic diseases that may cause SD [1–4]. We report the case of a postmortem examination integrated with molecular autopsy leading to the diagnosis of arrhythmogenic left ventricular cardiomyopathy (ALVC) due to a previously unreported desmoplakin (DSP) variant in a dead proband and in his living son, who received a defibrillator for SD primary prevention.

Case Report

A 49-year-old man died suddenly during physical activity. Personal and familial anamnesis was silent. The patient had undergone a routine pre-sport cardiologic evaluation – comprehensive of ECG and echocardiogram - the previous year at another Center and no abnormal findings were reported.

The heart was explanted in order to remove the valves for tissue banking, and then examined at our Cardiovascular Pathology Unit.

Macroscopic examination of the heart revealed moderate left ventricular (LV) dilatation (**Fig. 1B-E**). The LV subepicardial myocardial border was irregular and scalloped due to the presence of multiple fibro-fatty areas infiltrating/replacing the myocardium, mainly in the mid-basal antero-lateral (**Fig. 1B-D**) and apical infero-lateral walls (**Fig. 1E**). In the anterior septum an

area of fatty tissue originating from the subepicardium layer and progressing toward the mid-mural section was evident (**Fig. 1B-C**). The right ventricle (RV) was mildly dilated in the inflow tract, with a near-regular subepicardial border.

Histology findings were striking and confirmed the extensive and diffuse fibro-fatty replacement of the LV myocardium, originating from the subepicardial layer and spreading toward the mid-mural layer without any involvement of the subendocardial layer; the infero-lateral wall showed the most extensive fibro-fatty replacement. Fibro-fatty replacement also involved the mid-basal anterior septum while the RV had only mild fatty infiltration with no significant fibrosis. The myocardium showed severe myocell alterations: increased size (**Fig. 2C**), attenuation (**Fig. 2D**), vacuolization, irregular shaped nuclei (**Fig. 2E-F**). No inflammation was found. At histology the coronary arteries had only mild subintimal hyperplasia.

Pathology diagnosis was left dominant arrhythmogenic cardiomyopathy.

Genetic testing with Next Generation Sequencing was performed on DNA isolated from explanted heart paraffin samples (174 candidate genes for channelopathies and cardiomyopathies were analyzed, **Supplementary material**). Two variants of interest were identified in genes associated with arrhythmogenic cardiomyopathy (AC), both confirmed by Sanger sequencing: a heterozygous mutation c.1150C>G (p.Leu384Val) in exon 12 of the TMEM43 (transmembrane protein 43) gene, and a heterozygous mutation c.3533 T>G (p.Leu1178Arg) in exon 23 of the DSP (desmoplakin) gene. **Table 1** summarizes the cytogenetic and genomic locations of the two variants, their frequency in the gnomAD and Exon Variant Server databases and the prediction of their impact according to four different algorithms. The available information about the DSP variant can be classified following the criteria set by the American College of Medical Genetics and Genomics (ACMG) Standards and Guidelines [5]; this results in the following evidences of pathogenicity: two moderate (a-

absent in population databases and b – novel missense change at an amino acid residue where a different pathogenic missense change has been seen before [6]) and three supporting evidences (a- in silico prediction of deleterious effect, b – co-segregation with disease in multiple family members, c – patient’s phenotype highly specific for gene involved). On this basis, the DSP variant must be considered “Likely Pathogenic” (2 Moderate and ≥ 2 Supporting evidences). Evaluation of the TMEM43 variant by the same criteria leads to no definitive classification, therefore its status is confirmed as variant of unknown significance (VUS).

A targeted familial screening was activated. Both parents of the proband died of natural causes while the only brother is living abroad and is unavailable for clinical/genetic evaluation. The proband had one child, a 19-year-old asymptomatic boy who underwent cardiac screening. ECG and echocardiography were normal (**Fig.3**). Cardiac magnetic resonance (CMR) showed normal biventricular volumes and function without any regional abnormalities. At T2 weighted analysis a hypointense area was identified in the LV infero-lateral segment. On post contrast sequencing a long mid-mural stria of late gadolinium enhancement (LGE) was identified in the infero-lateral wall both in 4 chambers and in short axis images (**Fig.3**). Diagnostic work-up was completed with an exercise stress test and a Holter monitoring, which were unremarkable; in particular, no arrhythmias were recorded. Genetic testing was performed and only the DSP variant was detected. Therefore, the young patient was diagnosed with ALVC. Given the history of sudden cardiac death in a first-degree relative, the possibility of defibrillator implantation for SCD primary prevention was thoroughly discussed with the boy and his mother, clearly explaining the lack of solid evidence in this context [7] as well as the benefits and the risks of such strategy. They both expressed a strong opinion in favor of the implantation and this factor importantly influenced the final decision. As a result, the young patient gave his informed consent to the implant of a subcutaneous defibrillator.

Discussion

Our case report highlights the utility of a regional network with a diagnostic pathway specifically dedicated to sudden death victims where clinicians, cardiovascular pathologists and geneticists cooperate in order to identify the cause of death and activate a targeted familial screening.

We reported the case of a middle-aged man who died suddenly during moderate physical activity, and whose heart was centralized to our Institution as part of a valve tissue banking regional protocol. The histopathological examination of the heart revealed abnormalities suggestive of arrhythmogenic left ventricular cardiomyopathy; genetic testing results, albeit not conclusive, supported this hypothesis.

Clinical and genetic evaluation of the only son identified CMR signs suggestive of the same disease (ALVC) leading to the final decision of a subcutaneous defibrillator implantation for SCD primary prevention.

Left dominant arrhythmogenic cardiomyopathy is the most rare and recently described AC variant. Precise diagnostic guidelines are still lacking and this cardiomyopathy is probably clinically under-recognized as the abnormal LV myocardial substrate not necessarily causes contractile dysfunction or electrocardiographic abnormalities [8,9].

The relationship between SD and left ventricular involvement in AC has been recently investigated in detail [10]. In fact, in a large cohort of 202 SCD victims with a histopathological diagnosis of AC, authors reported a biventricular disease in 141 hearts (70%) and an isolated left ventricular involvement in 35 cases (17%). In our case SD was the first clinical expression of the disease in the absence of previous symptoms or medical records. This should not be surprising given the non-transmural extension of the pathological process as well as the limited

circumferential distribution. On the other hand, also the son's ECG and echocardiogram were normal but a CMR revealed LGE aspects highly specific for ALVC, with a topographic distribution that matched the histopathological abnormalities found in the father [11]. Therefore we speculate that without such a thorough post-mortem evaluation, the boy would have been considered healthy with potentially tragic implications [12–14].

The proband carried two variants of interest in genes associated with AC [15]. Importantly, the DSP variant - exon 23, c.3533T>G (p.Leu1178Arg) - was detected also in the living son. Whereas the causative link between several DSP variants and ALVC is well established [16–18], the pathogenic role of TMEM43 mutations has been poorly investigated. In fact, to date, the few described TMEM43 variants have been detected exclusively among patients with arrhythmogenic right ventricular cardiomyopathy while the role in ALVC is still unclear [19–23]. On the basis of the available information, the TMEM43 c.1150C>G p.Leu384Val variant is a VUS since it is not possible to assign it to one of the ACMG categories. On the other hand, the DSP variant is “likely pathogenic” according to the ACMG Guidelines; in particular, we noted its absence in public databases of human genetic variation, such as gnomAD - consisting of 125,748 exomes and 15,708 genomes to date – and the fact that a variant involving the same amino acid in DSP was reported in a family with AC. It is also relevant that the DSP gene has only two protein-coding transcripts reported in ENSEMBL (ENST00000379802.8 and ENST00000418664.2) and in both cases the p.Leu1178 amino acid is present in the resulting protein [5,6].

As genetic testing of AC enters standard care it is likely that the status of either or both variants will be confirmed or updated; it should also be noted that formally we cannot exclude a digenic contribution (with TMEM43 as modifier) to the genesis of SCD [24,25].

In our case the opportunity of a defibrillator implantation for SCD prevention was thoroughly discussed. Currently, it is impossible to stratify the risk of major arrhythmic events for patients with ALVC due to the scarce evidence, derived mainly from case reports and small series.

Therefore, in this scenario, the possibility of a defibrillator implantation should be considered on a case-by-case basis, taking into account the psychological impact as well as the patient's preference.

"Hic mors gaudet succurere vitae: here death is delighted to aid life" is the classic plaque exhibited in many anatomical theatres around Europe [26,27]. Our case report embodies a model of multidisciplinary approach to sudden death where an accurate cardiopathological post-mortem examination was implemented by molecular testing leading to the diagnosis of a rare hereditary disease, subsequently detected also in the proband's living son as a result of a targeted familial screening.

Funding sources: "Fondazione Luisa Fanti Melloni", University of Bologna, Italy.

Table 1. Features of the variants identified in this study in the TMEM43 and DSP genes

Gene and Variant	Cytogenetic location, genomic position (GRCh38) and Ref.Seq.	MIM phenotype	gnomAD frequency	Exome Variant Server	SIFT	Poly Phen	CADD	Mutation Taster
TMEM43 c.1150 C>G p.Leu384Val	3p25.1 3:14183242C>G NM_0243342	Arrhythmogenic Right Ventricular Dysplasia Familial 5; ARVD5 [#604400]	3.19e-5	absent	tolerated 0.96	benign 0.003	7.57	Poli-morphism
DSP c.3533 T>G p.Leu1178Arg	6p24.3 6:7579723T>G NM_004415.4	Arrhythmogenic Right Ventricular Dysplasia Familial 8; ARVD8 [#607450]	absent	absent	deleterious 0.01	probably damaging 0.996	26.9	disease causing

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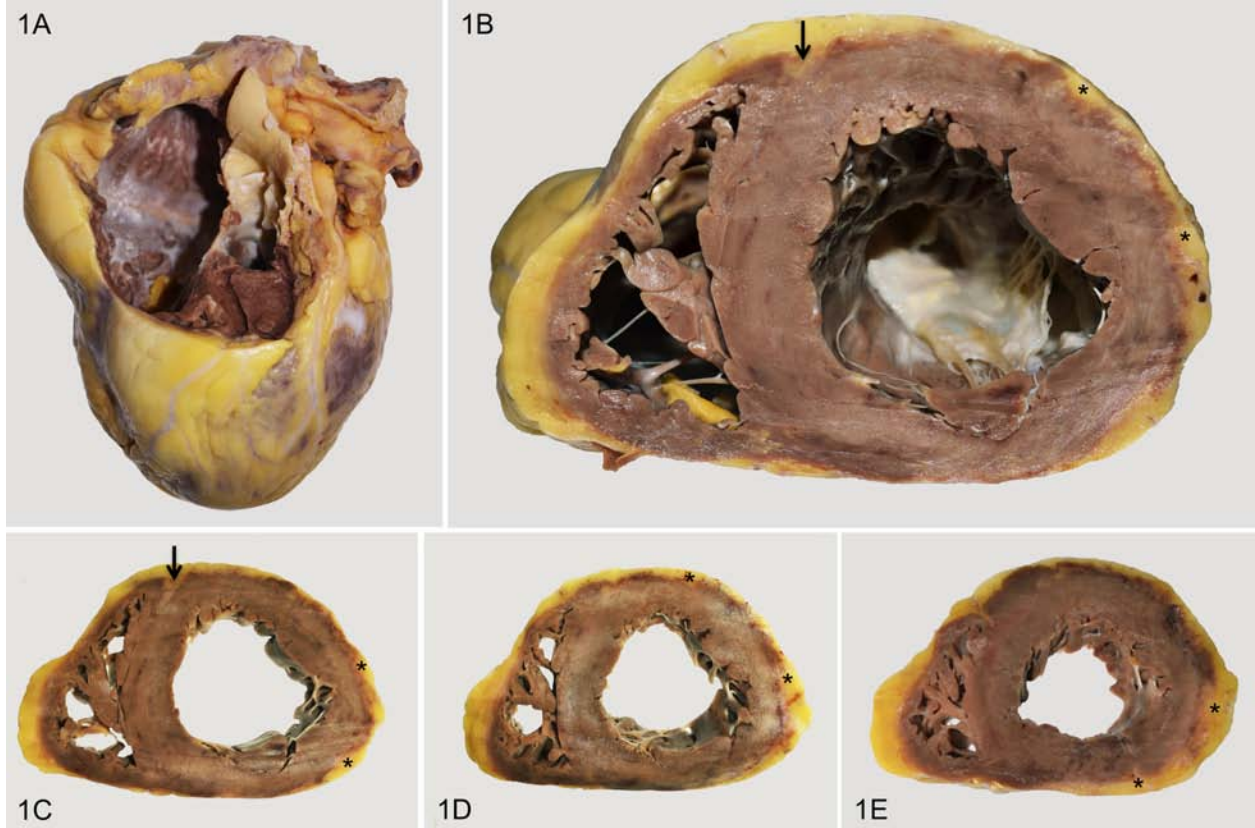
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FIGURE LEGENDS

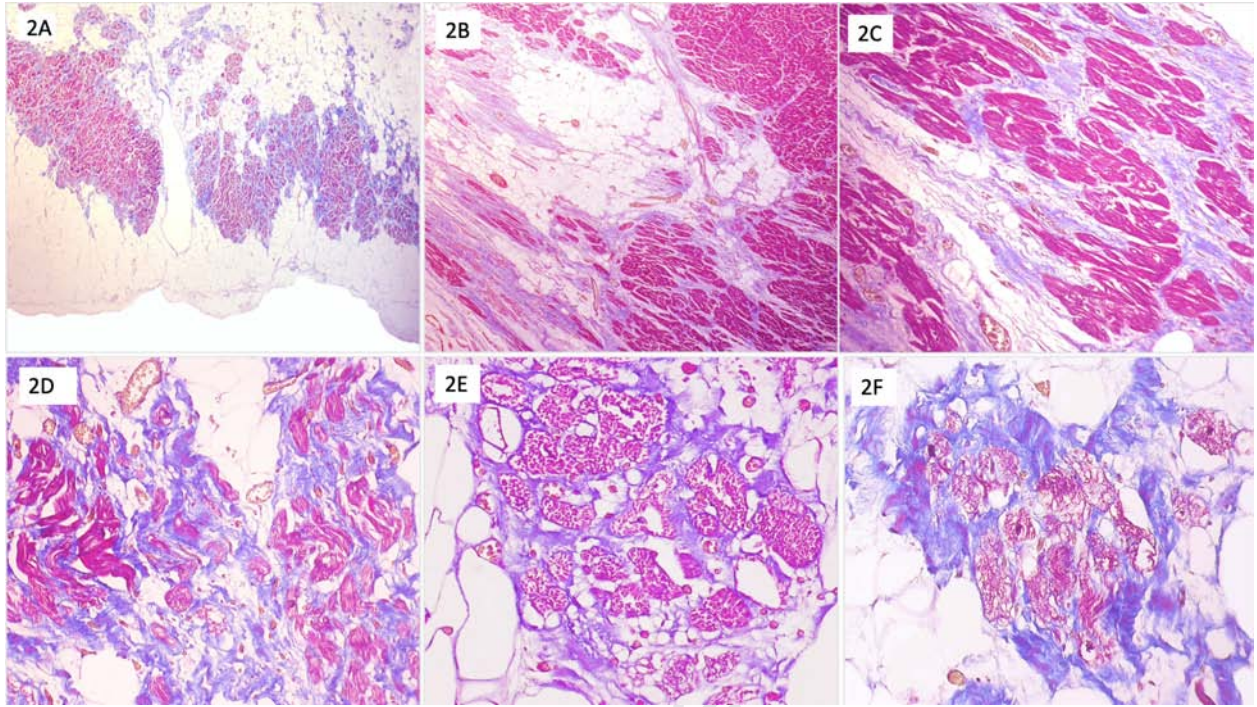
Fig. 1. Macroscopy of the heart. Heart is mildly dilated and altered in shape due to rounded apex (1A). On short-axis slices obtained from the apex to the mid-ventricular level, the presence of fibro-fatty areas infiltrating/replacing the myocardium makes the LV subepicardial myocardial border irregular (asterisks). A layer of fatty tissue progressing from the subepicardium towards the mid-mural area is evident in the anterior septum too (arrows).

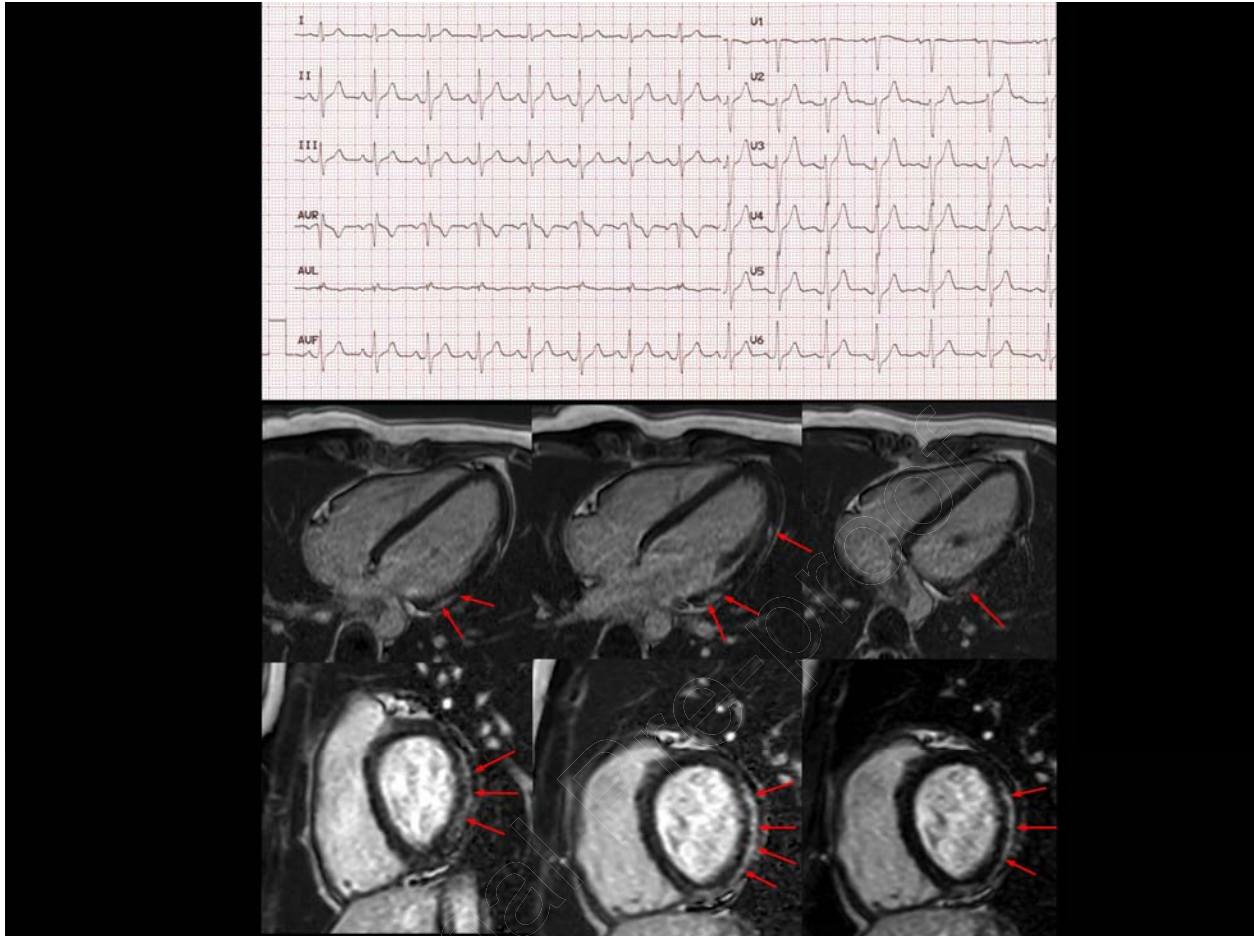
Fig. 2. Histology of the heart. Dense fibrosis is admixed to fatty tissue and to residual myocardium (A-B, Azan Mallory trichrome, 50x; 100x) and severe myocyte alterations are evident: increased size (C, Azan Mallory trichrome, 100x), attenuation (D, Azan Mallory trichrome, 200x) and cytoplasmic vacuolization (E-F, Azan Mallory trichrome, 400x).

Fig.3. Instrumental tests of the 19-year-old proband's son. Normal ECG. Linear LGE in subepicardial-midwall region of the left ventricular infero-lateral wall.



Journal





HIGHLIGHTS

- A multidisciplinary network for sudden death victims increases the diagnostic yield
- Autopsy combined with genetic testing may identify rare inherited cardiac diseases
- Identifying the cause of death is essential to guide a targeted familial screening

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