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Images in cardiology

Genetic modifiers to the PLN L39X mutation in a patient with DCM and sustained ventricular tachycardia?

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INTRODUCTION

Idiopathic dilated cardiomyopathy (DCM) is a leading cause of heart failure characterized by an enlarged ventricular cavity leading to systolic dysfunction. DCM patients have a considerable annual mortality rate of 5–10%, with half of them being sudden unexpected deaths due to ventricular tachycardia (VT) or ventricular fibrillation (VF).¹ Although a multifactorial disease, DCM appears to be inheritable in approximately 70% of cases.^{2,3} Causative gene mutations have been identified in a broad range of genes coding for proteins with a variety of function, such as cytoskeletal, sarcomeric or ion homeostasis related.⁴ Among the latter category, several mutations have been identified in Ca²⁺ handling proteins in familial and sporadic DCM cases. An increasing body of evidence indicates that abnormal intracellular Ca²⁺ handling underlies contractile dysfunction^{5,6} and contributes to ventricular arrhythmogenesis in failing myocardium.^{7,8} A prime example is phospholamban (PLN), which is directly involved in the uptake of Ca²⁺ by the sarcoplasmic reticulum (SR), on a beat-to-beat basis, thereby regulating cardiac contraction and relaxation. PLN mutations have been directly associated with the development of dilated cardiomyopathy and heart failure in patients and animal models.⁹ However, modifier genes are thought to influence the clinical outcome both in PLN cases, as well as DCM cases in general.¹⁰ We herein describe a DCM case which illustrates the complex genetic contribution to disease development and progression.

CLINICAL REPORT

Clinical characteristics

A male patient aged 56 years presented for evaluation because of sustained ventricular tachycardia episodes (Figure 1). He had a history of dilated cardiomyopathy diagnosed at the age of 40 years. Patient also presented heart failure symptoms (NYHA class II) with left ventricular ejection fraction (LVEF) of 25%. The ECG showed atrial fibrillation with frequent ventricular extra systolic beats (Figure 2). The Echo revealed severe left ventricular dilatation and systolic dysfunction (Figure 3). An AICD was implanted and the patient presented several ventricular tachycardia episodes terminating by AICD firing (Figure 4) during the following years. Patient had also presented clinical deterioration with advance heart failure symptoms and frequent hospital admissions especially 2-3 years after his presentation. He finally died because of end stage heart failure at the age of 60.

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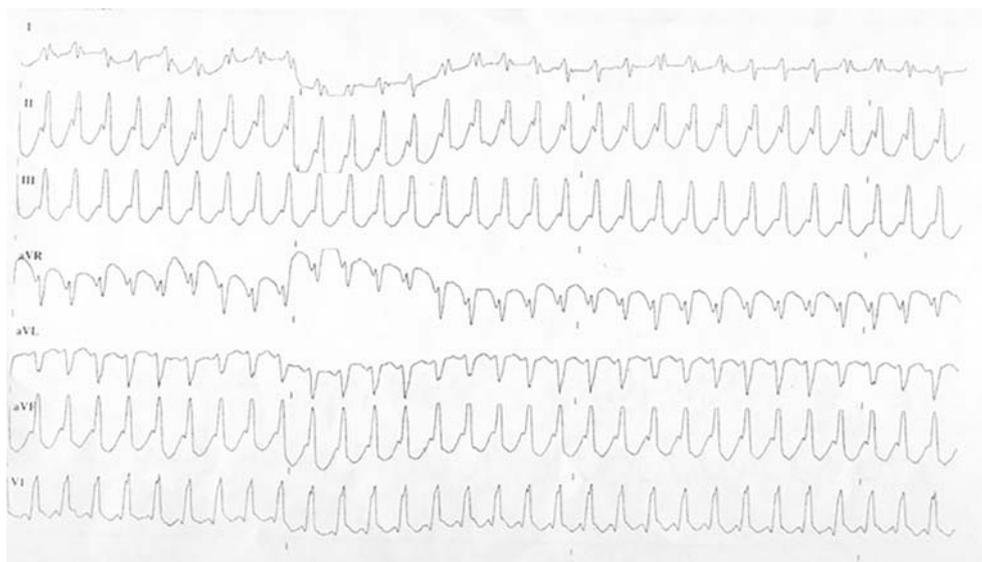


Figure 1. Sustained monomorphic ventricular tachycardia episode recorded in patient via ECG.

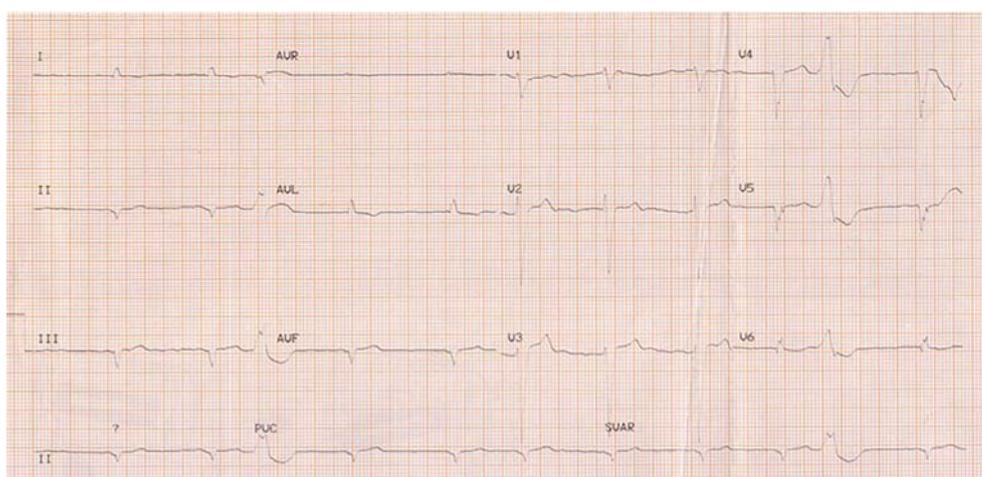


Figure 2. Patient ECG revealing atrial fibrillation with frequent ventricular extra systolic beats.

Genetics characteristics

High quality DNA was extracted from the peripheral blood of the patient, and was analysed by Next Generation Sequencing using the HiSeq Illumina platform. One hundred and seventy genes previously associated with heart disease or known to be involved in cardiac function were screened, and 163 genetic variants were detected in this patient. Among these, the vast majority were predicted to be benign variants. However, there were three heterozygous variants of interest:

- (i) a known pathogenic nonsense mutation (c.116T > G) in the PLN gene, that leads to a premature stop codon (L39X) (Figure 6).⁹
- (ii) a frameshift mutation (c.1495_1496insAGAC) in the C-terminus of CACNB2 (the beta subunit of the voltage-dependent calcium channel Ca(v)1.2) (Figure 6).
- (iii) a non-synonymous single nucleotide polymorphism (SNP) (c.9217C > T; p.L3073F) in laminin 2 (LAMA2), predicted to have a deleterious according to the Sorting Intolerant From Tolerant (SIFT) bioinformatical algorithm, and a possibly damaging effect according to the Polymorphism Phenotyping v2 (Polyphen2) algorithm (Figure 6).
- (iv) a non-synonymous SNP (c.6082A > G; p.T2028A) in the Alstrom Syndrome 1 (ALMS1) gene, predicted to have a deleterious effect according to SIFT.

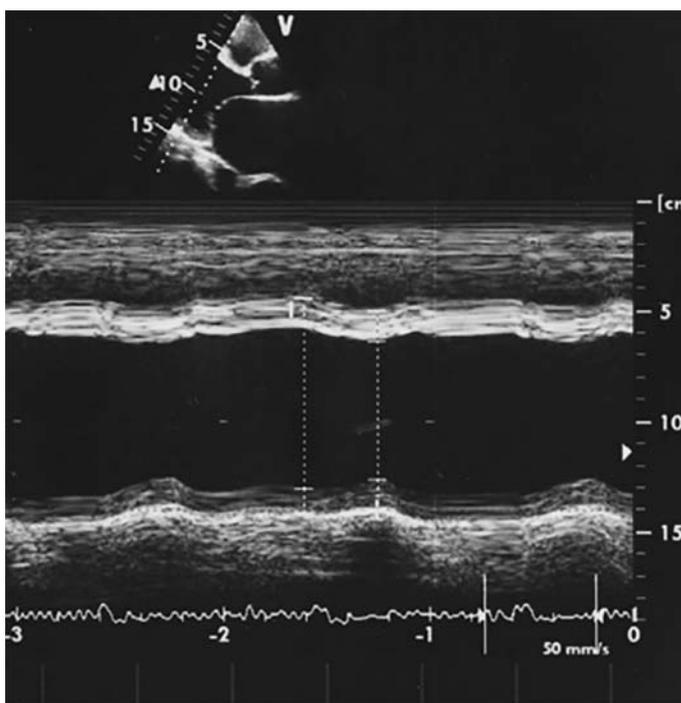


Figure 3. Patient echo study revealing severe left ventricular dilatation (left ventricular end diastolic diameter: 70 mm and left ventricular end systolic diameter 59 mm) and severe systolic dysfunction (left ventricular ejection fraction: 25%).

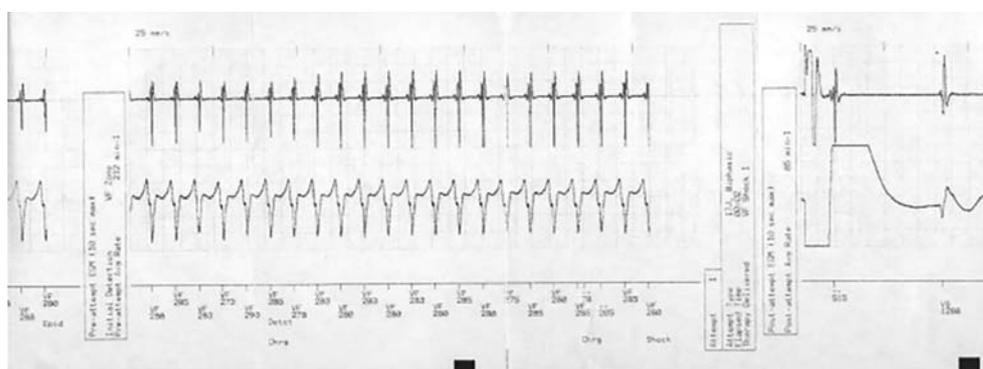


Figure 4. Patient AICD record revealing ventricular tachycardia episode that terminated by AICD firing.

All four genetic variants were confirmed by targeted Sanger sequencing. High-throughput sequencing is emerging as a powerful approach in revealing genotype-phenotype correlations of clinical significance.¹¹

DISCUSSION

Calcium homeostasis plays a critical role in normal cardiac function, and its impairment can lead to DCM. The sarcoplasmic reticulum (SR) is the principal organelle that controls intracellular Ca^{2+} cycling in cardiomyocytes, and thereby regulates cardiac contraction and relaxation. For the cardiomyocyte to be in a steady state with respect to intracellular Ca^{2+} balance, the amount of Ca^{2+} released from the SR into the cytoplasm must equal that re-accumulated by the action of SERCA uptake pumps. Cytosolic Ca^{2+} is sequestered into the SR lumen by the Ca^{2+} -ATPase (SERCA2a) during muscle relaxation. The stored Ca^{2+} is subsequently released from the SR through the ryanodine receptor channels to activate myofibrillar contraction. The activity of SERCA2a is reversibly regulated by PLN, a 52 amino acid phosphoprotein (Figure 5 and 6A). Dephosphorylated PLN interacts with SERCA2a and decreases the

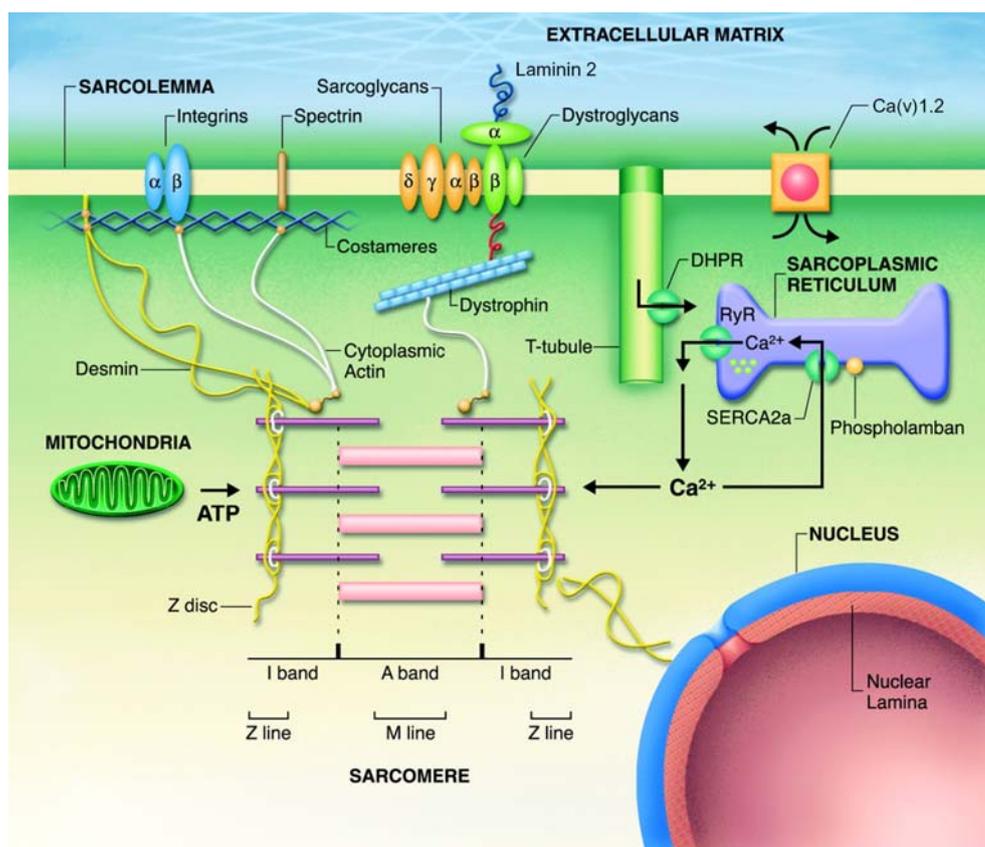


Figure 5. Schematic of the cardiomyocyte structure components, including PLN, laminin 2 and Ca(v)1.2 (modified from Fatkin et al Phys Rev 2012).

affinity of the Ca^{2+} -pump for Ca^{2+} , whereas phosphorylation of PLN through the beta-adrenergic pathway relieves this inhibition and augments relaxation.¹² It has been postulated that phosphorylation of PLN at both S16 and T17 by PKA and CaMKII activation is the ultimate goal of sympathetic stimulation in heart, and PLN mutations can be causative for DCM.¹³ Homozygosity for the L39X mutation in PLN has been shown to lead to reduced PLN mRNA expression and absence of the PLN protein.⁹

The phenotype of L39X mutation carriers in the PLN gene has been shown to vary considerably, ranging from severe DCM to rare reports of hypertrophic cardiomyopathy (HCM) or even normal cardiac function.^{9,14} In cardiomyopathies there are several reports of different mutations in a gene being

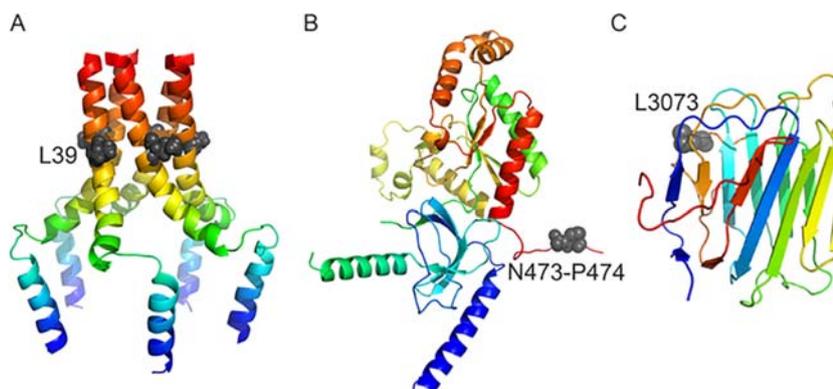


Figure 6. Protein structures and positions of mutations. A) The pentameric structure of PLN. B) A partial modelled structure of CACNB2 (residues 88 to 474). C) A model of the fifth module of laminin G-like domain. The 3D structures are represented as coloured cartoons, where the grey spheres indicate the key mutant residues.

a nonsense mutation in exon 31 (c.4645 C > T, p.Arg1549Stop). In our patient, the key residue was located in Laminin G-like 5 domain (p. 2939 to 3120). Since there is no crystal structure available for this region, we modelled the domain in DS using a template structure of the fifth laminin g-like module of the mouse laminin alpha2 chain.²⁷ The sequence of the mouse and human shared 88% identity and importantly the key residue L3073 was conserved (see sequence alignment, Figure 7B). The modelled domain was optimized by energy minimization using CHARMM force field in DS (Figure 6C).

Mutations in ALMS1 lead to Alström syndrome, a rare autosomal recessive genetic disorder characterized by metabolic, endocrine and sensory impairment (blindness and deafness) as well as liver, pulmonary and renal disease, over time.²⁸ Approximately 60% of ALMS cases develop DCM, while recently two siblings presented with central conduction system disease and cardiac rhythm abnormalities.²⁹ Although there are no reports on the role of ALMS1 in the heart, it is interesting to note that ALMS1 silencing in kidney epithelial cells inhibited intracellular calcium influx.³⁰

Overall, the combination of the CM causative L39X mutation in PLN, with the predicted pathogenic genetic variants in CACNB2, LAMA2 and ALMS1 in DCM is associated with sustained VT.

WHAT HAVE WE LEARNED?

- This is the first report of a L39X mutation carrier presenting with sustained VT, in addition to cardiomyopathy.
- These observations strengthen the evidence of one mutation contributing to multiple different phenotypes, possibly under the influence of other genetic or environmental factors.
- Next generation sequencing is a powerful, unbiased, spherical approach to depict genetic variants with a causative or modifying role.
- Three modifier gene candidates emerge that could be implicated in the development of sustained VT in DCM patients, and specifically carriers of the PLN L39X mutation.

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