***Supplementary material***

*Zienciuk-Krajka A, Chmara M, Lica-Gorzynska M, et al. The novel pathogenic variant in the LMNA gene in a four-generation family with sudden deaths and cardiomyopathy: utility of molecular autopsy. Kardiol Pol. 2021.*



## Imaging

Participants underwent echocardiography using a GE VIVID9 ultrasound system (GE Ultrasound, Horten, Norway) equipped with a phased-array transducer (M5S). Standard echocardiographic parameters were obtained according to the principles described in the recommendations [1]. Left atrial (LA) and left ventricular (LV) volumes were measured using biplane methods as averaged values from four- and two-chamber views. LA volume was indexed by body surface area. Left ventricular ejection fraction was measured using biplane Simpson's method [1]. The genotype positive family members underwent cardiac magnetic resonance imaging on 1.5T scanner (Aera, Siemens, Erlangen, Germany) or 3T scanner (Achieva, Phillips Healthcare, Best, the Netherlands). On cardiac magnetic resonance imaging, a series of morphofunctional parameters were carefully evaluated. Standard volumes and function parameters were measured based on long-axis cine, and a short-axis cine stack covering the entire LV. The presence of LGE was visually assessed independently by two experienced readers based on the 17 segment model [2]. Any discrepancy was solved by consensus.

## Mutational analysis

Genomic DNA was isolated from peripheral blood leukocytes using the chemagic DNA Blood Kit special and chemagic 360 instrument (PerkinElmer). Mutational analysis of the index patient was performed using PED MASTR Plus assay (Agilent) and MiSeq (Illumina). Bioinformatic analysis was conducted using SeqNext (JSI Medical Systems) and Alamut Visual software (Interactive Biosoftware). All identified variants were classified as recommended by the American College of Medical Genetics and Genomics guidelines [3]. The presence of a detected variant in exon 2 of *LMNA* gene was confirmed by bidirectional Sanger sequencing. Cascade screening of FMs was also performed by Sanger sequencing. Finally, for the purpose of a molecular autopsy of patient II-9, DNA was isolated from archival FFPE tissue using DNA sample preparation kit (Roche).

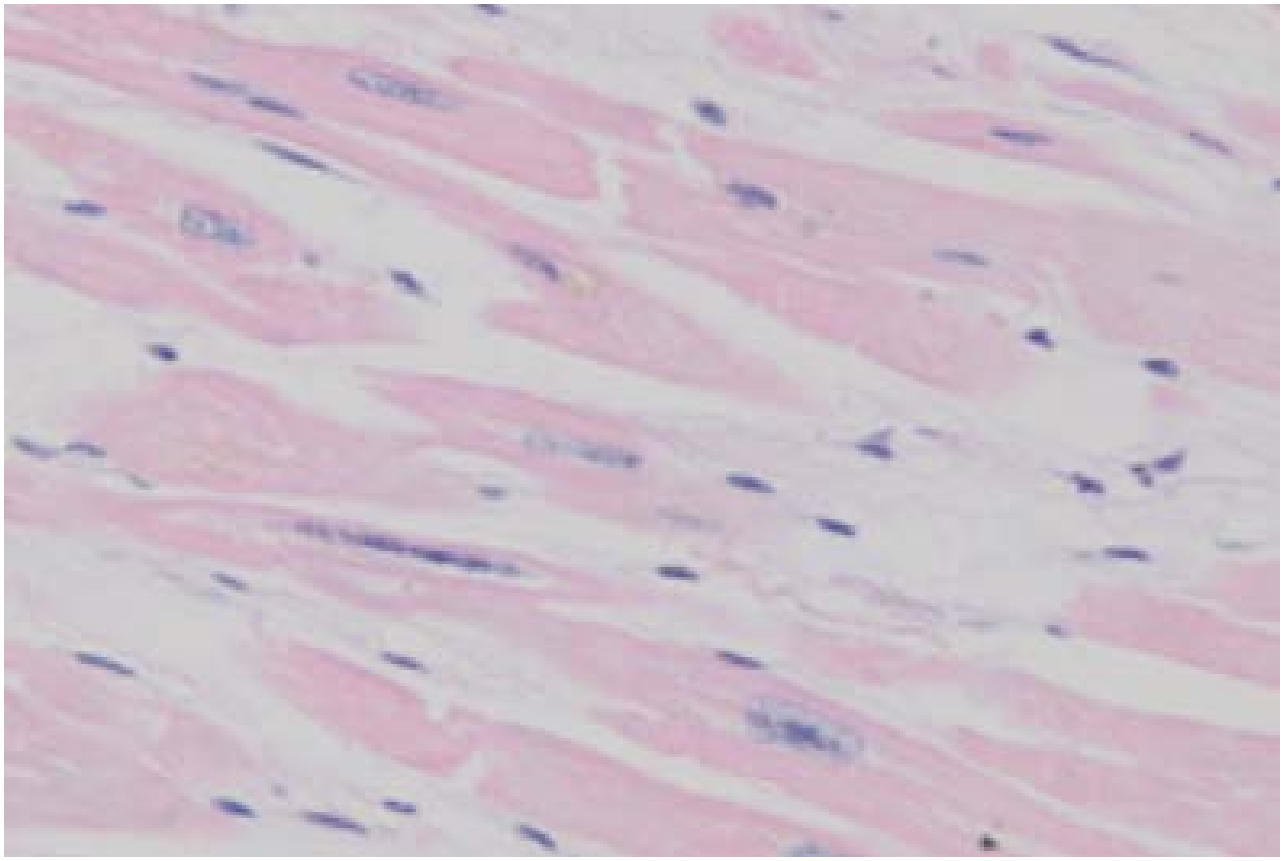

**Figure S1.** Microscopic view of cardiac specimen obtained from the autopsy of the patient II-9 showing myocardial hypertrophy and degeneration with atypical nuclei of cariomyocytes

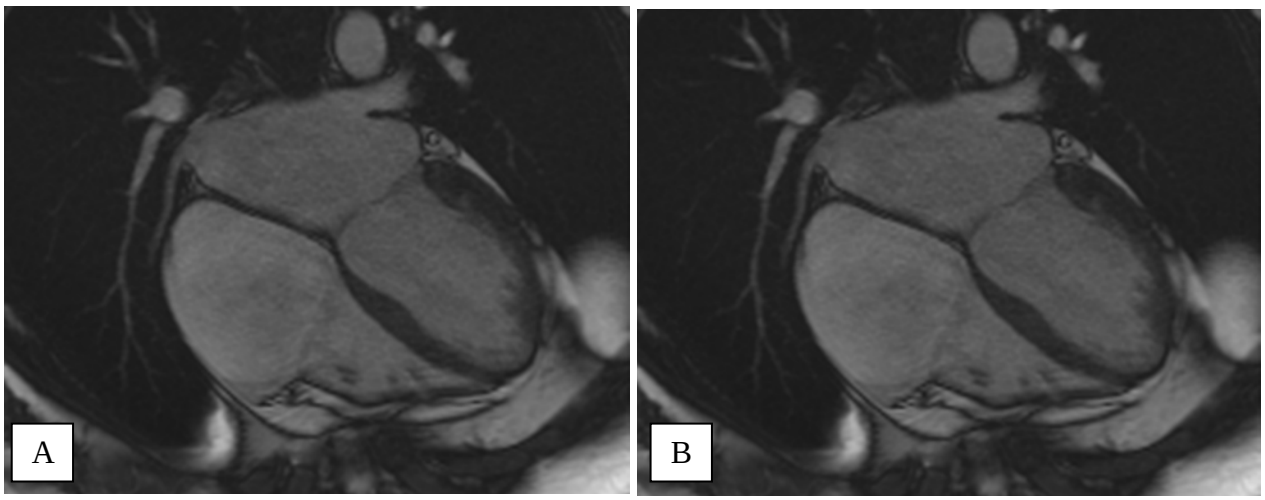


**Figure S2.** Long axis (4CH) systolic (**A**) and long axis (4CH) diastolic (**B**) still frames from routine balanced steady state free precession cine sequence in a 43 years old female member of the proband's family, showing slight biatrial and left ventricular enlargement (LVEDVI of 120 ml/m$^2$), relative thinning of compacted layer in several LV segments. LVEF was moderately reduced at 45% (decreased in comparison to CMR 3 years prior, where LVEF was 53% and LVEDVI 102 ml/m$^2$) (Siemens Aera 1.5T, Erlangen, Germany).

Abbreviations: 4CH, 4-chamber view; CMR, cardiac magnetic resonance; LV, left ventricular; LVEDVI, LV end-diastolic volume index; LVEF, LV ejection fraction

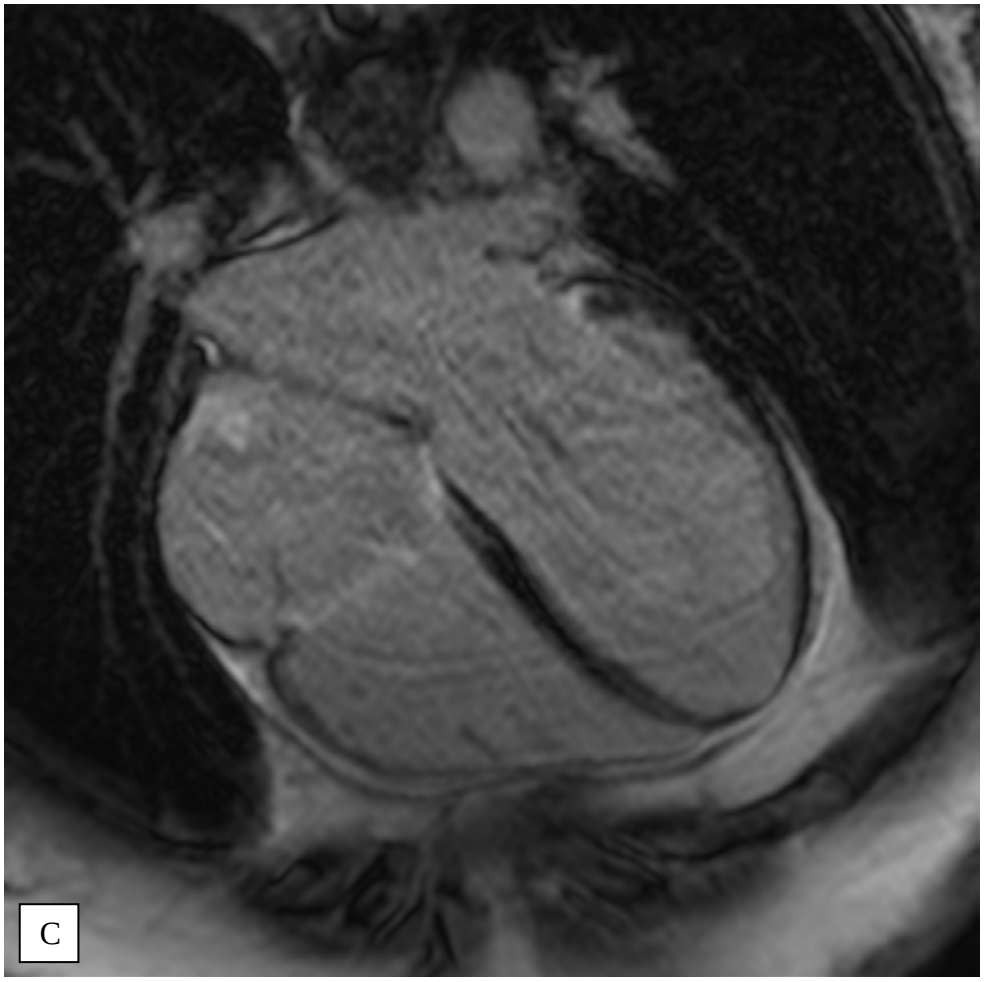


**Figure S2. C.** Example of late gadolinium enhancement (LGE) extent and distribution in the same patient with a *LMNA* mutation as in panel A. Minimal, intramural LGE in basal-infero-septal segment, discrete streak of LGE in the mid- and apical portions of the septum. Images acquired with a phase-sensitive inversion recovery sequence (Siemens Aera, 1.5T Erlangen, Germany)

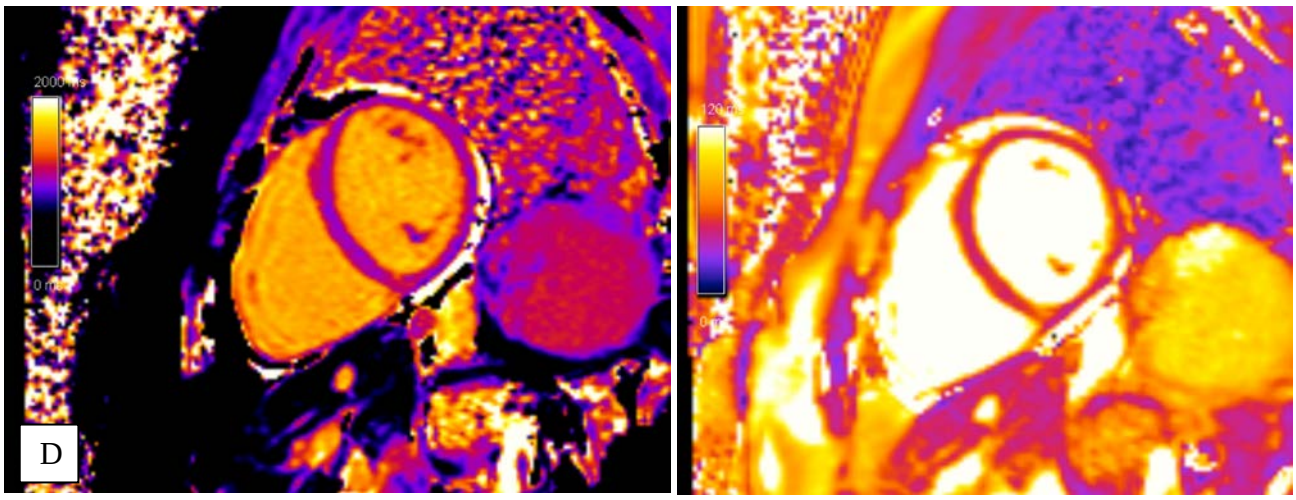


**Figure S2. D.** Parametric mapping of the basal short axis slice showing mildly increased native T1 time (Left; 1060 ms; ref range: 993 ± 21 ms; 951–1035 ms) and normal T2 time (Right; 46 ms; ref range: 44 ± 2.4 ms; 39–49 ms) in the same patient with a *LMNA* mutation as in panel A. This configuration together with mildly elevated ECV of 35% is suggestive of interstitial fibrosis of the myocardium, in the absence of acute injury. Images acquired with a MOLLI sequence (T1) and bSSFP sequence (T2). (Siemens Aera, 1,5T Erlangen, Germany).

Abbreviations: bSSFP, balanced steady-state free precession; ECV, extracellular volume; MOLLI, modified Look-Locker Inversion

**Table S1.** Sudden cardiac death risk factors in genotype-positive patients with p.(Arg166Glyfs*11) *LMNA* variant

| Initials | Patient pedigree number | Age, years | Symptoms | Sex | LVEF, % | nsVT | AVB I | Higher degree AVB[a] | *LMNA*-risk VTA score, %[b] | ESC SCD-risk score [c] | Creatine kinase (n < 168) | BNP | CMR LGE | Medication |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **S-I** | **II-7** | **44** | **Exercise intolerance palpitation** | F | 45 | 10 beats 120 bpm | 1 | 0 | 17.0 | 3 | — | — | Mid-myocardial enhancement in basal IVS | ACEI |
| **Z-D** | **II-8** | **41** | **Exercise intolerance palpitation** | F | 42 | 1× triplet 136 bpm | 0 | 0 | 17.9 | 3 | 102 | 24 | Mid-myocardial enhancement in IVS and subepicardial LGE in basal and posterior LV segment | BB, ACEI |
| **K-A** | **III-1** | **30** | **None** | F | 50 | 0 | 0 | 0 | 7.5 | 1 | 472 | 20 | No | None |
| **L-M** | **III-3** | **36** | **None** | F | 59 | 0 | 1 | 0 | 14.3 | 1 | 224 | 10 | No | None |
| **S-K** | **III-14** | **21** | **None** | F | 0 | 0 | 0 | 0 | 6.9 | 1 | 239 | 10 | No | None |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **S-P** | **III-15** | **19** | **None** | F | 0 | 0 | 0 | 0 | 6.6 | 1 | 524 | 10 | Mid-myocardial enhancement in basal IVS | None |
| **Z-M** | **III-17** | **16** | **None** | M | 57 | 0 | 0 | 0 | 10.8 | 2 | - | - | Not done | None |

[a]Higher degree AVB refers to second degree Mobitz 2 block and 3rd degree block; [b]see [4], [c]see[5]
Abbreviations: ACEI, angiotensin-converting-enzyme inhibitor; AVB, atrioventricular block; BB, beta-blocker; BNP, brain natriuretic peptide; CMR, cardiac magnetic resonance; ESC, European Society of Cardiology; IVS, interventricular septum; LGE, late gadolinium enhancement; LV, left ventricular; LVEF, left ventricular ejection fraction; nsVT, non-sustained ventricular tachycardia; SCD, sudden cardiac death; VTA, ventricular tachyarrhythmia

## REFERENCES


1. Lang R, Badano L, Mor-Avi V, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging. 2015; 16(3): 233–270, doi: 10.1093/ehjci/jev014, indexed in Pubmed: 25712077.
2. Cerqueira MD, Weissman NJ, Dilsizian V, et al. American Heart Association Writing Group on Myocardial Segmentation and Registration for Cardiac Imaging. Standardized myocardial segmentation and nomenclature for tomographic imaging of the heart. A statement for healthcare professionals from the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association. Circulation. 2002; 105(4): 539–542, doi: 10.1161/hc0402.102975, indexed in Pubmed: 11815441.
3. Richards S, Aziz N, Bale S, et al. ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015; 17(5): 405–424, doi: 10.1038/gim.2015.30, indexed in Pubmed: 25741868.
4. Wahbi K, Ben Yaou R, Gandjbakhch E, et al. Development and validation of a new risk prediction score for life-threatening ventricular tachyarrhythmias in laminopathies. Circulation. 2019; 140(4): 293–302, doi: 10.1161/CIRCULATIONAHA.118.039410, indexed in Pubmed: 31155932.
5. Priori S, Blomström-Lundqvist C, Mazzanti A, et al. 2015 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death. Eur Heart J. 2015; 36(41): 2793–2867, doi: 10.1093/eurheartj/ehv316, indexed in Pubmed: 26320108.