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CLINICAL RESEARCH

Early Mechanical Alterations in Phospholamban Mutation Carriers

Identifying Subclinical Disease Before Onset of Symptoms

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ABSTRACT

OBJECTIVES This study aimed to explore echocardiographic characteristics of phospholamban (PLN) p.Arg14del mutation carriers to investigate whether structural and/or functional abnormalities could be identified before onset of symptoms.

BACKGROUND Carriers of the genetic PLN p.Arg14del mutation may develop arrhythmogenic and/or dilated cardiomyopathy. Overt disease is preceded by a pre-symptomatic phase of variable length in which disease expression seems to be absent.

METHODS PLN p.Arg14del mutation carriers with an available echocardiogram were included. Mutation carriers were classified as pre-symptomatic if they had no history of ventricular arrhythmias (VAs), a premature ventricular complex count of <500/24 h, and a left ventricular (LV) ejection fraction of $\geq 45\%$. In addition, we included 70 control subjects with similar age and sex distribution as the pre-symptomatic mutation carriers. Comprehensive echocardiographic analysis (including deformation imaging) was performed.

RESULTS The final study population consisted of 281 PLN p.Arg14del mutation carriers, 139 of whom were classified as pre-symptomatic. In comparison to control subjects, pre-symptomatic mutation carriers had lower global longitudinal strain and higher LV mechanical dispersion (both $p < 0.001$). In addition, post-systolic shortening (PSS) in the LV apex was observed in 43 pre-symptomatic mutation carriers (31%) and in none of the control subjects. During a median follow-up of 3.2 years (interquartile range: 2.1 to 5.6 years) in 104 pre-symptomatic mutation carriers, nonsustained VA occurred in 13 (13%). Presence of apical PSS was the strongest echocardiographic predictor of VA (multivariable hazards ratio: 5.11; 95% confidence interval [CI]: 1.37 to 19.08; $p = 0.015$), which resulted in a negative predictive value of 96% (95% CI: 89% to 98%) and a positive predictive value of 29% (95% CI: 21% to 40%).

CONCLUSIONS Global and regional LV mechanical alterations in PLN p.Arg14del mutation carriers precede arrhythmic symptoms and overt structural disease. Pre-symptomatic mutation carriers with normal deformation patterns in the apex are at low risk of developing VA within 3 years, whereas mutation carriers with apical PSS appear to be at higher risk. (J Am Coll Cardiol Img 2020;■:■-■) © 2020 by the American College of Cardiology Foundation.

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ABBREVIATIONS AND ACRONYMS

CI = confidence interval

GLS = global longitudinal strain

HR = hazards ratio

IQR = interquartile range

LV = left ventricle/ventricular

LVEF = left ventricular ejection fraction

LVMD = left ventricular mechanical dispersion

PLN = phospholamban

PSS = post-systolic shortening

PVC = premature ventricular complex

RV = right ventricle/ventricular

RVFWS = right ventricular free-wall strain

VA = ventricular arrhythmia

Phospholamban (PLN) is a phosphoprotein in the sarcoplasmic reticulum that plays a key role in cardiac contraction and relaxation through regulation of calcium homeostasis in cardiomyocytes (1). Various cardiomyopathy-related mutations have been described in the gene that encodes PLN (2). One mutation of particular interest is p.Arg14del, which has been identified in a large subset of patients with arrhythmogenic right ventricular (RV) cardiomyopathy and dilated cardiomyopathy in the Netherlands (3). To date, this founder mutation is the most prevalent single cardiomyopathy-related mutation in the Netherlands (4). Besides the Netherlands, this mutation has also been identified in several other European countries and North America (5–9).

Family members of index patients with PLN p.Arg14del routinely undergo genetic cascade screening. This strategy has led to the identification of a large number of pre-symptomatic individuals (i.e., without phenotypic expression) who carry this mutation (10). Repeated cardiac screening is necessary in this group because of their risk of developing malignant ventricular arrhythmias (VAs) and/or heart failure (11,12). Disease penetrance seems to be age-dependent, with symptoms often beginning around the fifth decade (3,13,14). However, optimal follow-up frequency and timing of therapeutic intervention is still a major challenge in pre-symptomatic individuals because of incomplete disease penetrance and variable disease expressivity. Early identification of high-risk mutation carriers, even before the onset of symptoms, would enable close monitoring and early therapeutic intervention to prevent life-threatening VAs and/or slow down progression of heart failure.

A promising technique in this regard is echocardiographic deformation imaging. This noninvasive technique allows quantification of global and regional myocardial function and has previously been shown to reveal early signs of disease for several cardiomyopathies (15–20). Accordingly, we hypothesized that dysregulation of intracellular calcium handling in

PLN p.Arg14del mutation carriers leads to a change in myocardial mechanical behavior in early stages of disease, which can be detected by echocardiographic deformation imaging.

In the present study, we aimed to explore echocardiographic characteristics in PLN p.Arg14del mutation carriers to investigate whether structural and/or functional abnormalities could be identified before onset of symptoms. In addition, we aimed to evaluate the prognostic value of echocardiographic measurements in pre-symptomatic mutation carriers.

METHODS

This observational retrospective longitudinal cohort study was approved by the institutional medical ethics committee of the University Medical Center Utrecht and complied with the Declaration of Helsinki and the European General Data Protection Regulation.

STUDY POPULATION. Study participants were derived from a national registry in which all carriers of mutations associated with arrhythmogenic cardiomyopathy are enrolled, regardless of their phenotype (21). For the present study, we randomly included 350 PLN p.Arg14del mutation carriers (18 years or older) who underwent transthoracic echocardiography between 2006 and 2019 at the 1 of the 3 main including centers: University Medical Center Utrecht, University Medical Center Groningen, or Amsterdam University Medical Center. This group of mutation carriers consisted of both index patients and relatives. Subjects were excluded in case of an incomplete echocardiogram or an echocardiogram of suboptimal quality (explained in detail in the following). Subjects were also excluded in case they had other relevant cardiovascular disorders (Figure 1). If a subject had >1 available echocardiogram, the first one with appropriate image quality was used for this study.

In addition to mutation carriers, we included 70 control subjects. This control group consisted of: 1) healthy nonathlete subjects who were recruited for a previous study (22); and 2) subjects who were found to have no cardiovascular disease after comprehensive screening at the cardiology outpatient clinic.

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the JACC: Cardiovascular Imaging [author instructions page](#).

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FIGURE 1 Inclusion Flowchart

Phospholamban (PLN) p.Arg14del mutation carriers were derived from a national database. Mutation carriers were eligible for this study if they had an available echocardiogram of sufficient quality. The final study population included 281 mutation carriers, who were classified to the pre-symptomatic stage (n = 139), the arrhythmic stage (n = 91), or the structural stage (n = 51). LBBB = left bundle branch block; LVEF = left ventricular ejection fraction; PLN = phospholamban; PVC = premature ventricular complex; VA = ventricular arrhythmia.

CARDIAC EVALUATION

We first evaluated the medical history of PLN p.Arg14del mutation carriers until the echocardiographic examination with regard to: 1) occurrence of (non)sustained VA; 2) premature ventricular complex (PVC) count on Holter recordings; and 3) left ventricular ejection fraction (LVEF) measured by cardiac imaging (echocardiography or cardiovascular magnetic resonance imaging). Nonsustained VA was defined as ventricular tachycardia of at least 3 beats, with a frequency of at least 100 beats/min, lasting not more than 30 s. Sustained VA was defined as

ventricular tachycardia lasting >30 s, ventricular fibrillation, or appropriate implantable cardioverter-defibrillator intervention.

A cutoff value of 500 PVCs per 24 h was used according to the 2010 Task Force Criteria for arrhythmogenic right ventricular cardiomyopathy (23). A cutoff value of 45% was used for LVEF according to previous studies and an ongoing interventional trial (Phospholamban Related Cardiomyopathy Study - Intervention [i-PHORECAST]; NCT01857856), a cutoff value of 45% was used for LVEF (13,24). Based on these data, mutation carriers were classified into the following 3 groups: 1) pre-symptomatic stage: no

TABLE 1 Baseline Characteristics

	Controls (n = 70)	Pre-Symptomatic (n = 139)	Arrhythmic (n = 91)	Structural (n = 51)
Age (yrs)	33 (25–44)	33 (21–42)	49 (40–57)*	51 (41–64)*
Male	26 (37)	59 (42)	43 (47)	22 (43)
BSA (m ²)	1.8 (1.7–1.9)	1.9 (1.8–2.0)	2.0 (1.8–2.1)*	1.9 (1.8–2.1)
Proband	—	0 (0)	25 (27)	33 (65)
ARVC Dx	—	0 (0)	29 (32)	21 (41)
DCM Dx	—	0 (0)	0 (0)	43 (84)
VA history	—	0 (0)	57 (63)	40 (78)
ICD	—	0 (0)	26 (29)	34 (67)
Cardiac medication	—	9 (7)	53 (58)	50 (98)
ACEi/ARB	—	3 (2)	24 (17)	38 (27)
Antiarrhythmic	—	0 (0)	11 (8)	32 (23)
Beta-blocker	—	1 (1)	29 (21)	61 (44)
Diuretic	—	5 (4)	11 (8)	42 (30)
LVEF (%)	60 ± 4	58 ± 4	55 ± 5*	35 ± 10*
NYHA functional class ≥II	—	0 (0)	6 (7)	26 (51)
PVC count (n/24 h)	—	4 (1–55)	1,400 (814–3,849)	2,652 (1,111–5,135)
TWI in >1 lead	—	10/130 (8)	41/86 (48)	20/47 (43)
Low QRS voltages	—	16/133 (12)	36/89 (41)	37/50 (74)
LGE	—	19/78 (24)	23/42 (55)	6/7 (86)

Values are median (interquartile range), n (%), or mean ± SD, unless otherwise indicated. *Statistical significant difference in comparison to control subjects (i.e., Bonferroni adjusted $p < 0.05$).

ACEi = angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor blocker; ARVC = arrhythmogenic right ventricular cardiomyopathy; BSA = body surface area; DCM = dilated cardiomyopathy; Dx = diagnosis; ICD = implantable cardioverter-defibrillator; LGE = late gadolinium enhancement; LVEF = left ventricular ejection fraction; NYHA = New York Heart Association; PVC = premature ventricular complex; TWI = T-wave inversion; VA = ventricular arrhythmia.

history of VA, PVC count <500/24 h, and LVEF ≥45%; 2) arrhythmic stage: history of (non)sustained VA and/or PVC count ≥500/24 h and LVEF ≥45%; and 3) structural stage: LVEF <45%.

In mutation carriers who had no history of VA and a normal LVEF but no available Holter recording (until 1 month after the echocardiogram), we evaluated cardiac symptoms (palpitations or suspected cardiac [pre-]syncope) as reported by the treating physician. The absence or presence of these symptoms was used to classify these mutation carriers into the pre-symptomatic or into the arrhythmic stage, respectively.

In addition to the aforementioned data, we evaluated the New York Heart Association functional class as per the judgement of the treating physician. Standard 12-lead electrocardiograms that were recorded within 1 year before or after the echocardiogram were evaluated for the presence of low QRS voltages and T-wave inversions. Low QRS voltages were considered present when QRS peak-to-peak amplitudes in leads I, II, and III were <0.5 mV or when the amplitudes in all precordial leads were <1.0 mV. T-wave inversions were assessed in the precordial leads V₂ to V₆ and in the inferior leads II, III, and aVF, in the absence of a left or right bundle branch block. Contrast-enhanced cardiac magnetic

resonance studies that were performed within 1 year before or after the echocardiogram were categorized according to presence or absence of late gadolinium enhancement (LGE) (24).

CONVENTIONAL ECHOCARDIOGRAPHY. All echocardiograms were performed with Vivid 7, E9 and E95 machines (GE Healthcare, Horten, Norway) as part of routine clinical care. Echocardiograms were retrieved and uploaded to the University Medical Center Utrecht core laboratory. All measurements were performed by 1 experienced operator who was blinded to the clinical data during the measurements. Conventional echocardiographic measurements were performed in accordance with current recommendations (25). Details on these measurements are provided in the [Supplemental Appendix](#).

DEFORMATION IMAGING. Longitudinal strain analysis was performed by 2-dimensional speckle tracking with EchoPAC version 203 (GE Healthcare, Horten, Norway). More details on post-processing methods are provided in the [Supplemental Appendix](#) and in a previous study (26). As for the standard echocardiographic measurements, deformation analyses were performed by 1 experienced operator who was blinded to the clinical data during the measurements.

Global deformation imaging. For LV deformation imaging, apical 4-, 2-, and 3- chamber views were used

TABLE 2 Echocardiographic Measurements

	Controls (n = 70)	Pre-Symptomatic (n = 139)	Arrhythmic (n = 91)	Structural (n = 51)
LVEDV (ml/m ²)	54.2 (46.7–61.6)	53.8 (46.7–60.3)	55.6 (48.5–63.0)	76.2 (62.4–102.3)*
LVESV (ml/m ²)	21.4 (18.4–25.2)	21.3 (17.6–25.6)	25.5 (20.4–29.9)*	47.7 (36.5–72.2)*
LVEF (%)	59.8 ± 4.4	58.3 ± 4.2	54.5 ± 5.2*	35.0 ± 9.6*
RVOT-PLAX (mm/m ²)	15.4 (14.2–16.7)	14.9 (13.8–16.4)	16.9 (15.4–17.9)*	19.4 (16.6–23.1)*
RVOT-PSAX (mm/m ²)	16.0 (14.8–17.6)	15.8 (14.1–17.5)	17.3 (15.8–18.6)	20.5 (17.0–22.8)*
RVEDA (cm ² /m ²)	9.4 (8.4–10.5)	9.4 (8.3–10.4)	10.1 (8.5–12.0)	13.4 (11.5–15.8)*
RVESA (cm ² /m ²)	4.8 (4.4–5.9)	5.0 (4.3–5.8)	6.1 (4.8–7.3)*	10.1 (7.6–11.7)*
RV FAC (%)	46.8 ± 4.8	46.1 ± 5.5	41.3 ± 7.9*	28.8 ± 7.6*
LAVI (ml/m ²)	28.7 ± 6.8	27.7 ± 6.2	30.4 ± 7.7	41.3 ± 13.3*
E-wave velocity (cm/s)	81.0 (70.6–90.3)	78.7 (66.0–89.0)	64.5 (51.3–80.0)*	61.2 (50.1–74.3)*
A-wave velocity (cm/s)	44.8 (39.4–56.8)	51.0 (42.0–59.0)	53.3 (39.0–65.0)	49.9 (35.5–62.3)*
E-wave DT (ms)	175 (161–199)	181 (155–203)	203 (176–232)*	157 (141–202)*
Average E velocity (cm/s)	14.5 (11.5–15.8)	13.5 (10.5–16.0)	9.5 (8.0–12.0)*	6.0 (4.5–8.0)*
E/E ratio	5.9 (4.9–6.4)	5.9 (5.1–6.9)	6.3 (5.3–7.8)	9.9 (6.9–11.8)*
GLS (%)	21.5 ± 1.8	19.9 ± 1.9*	18.4 ± 2.1*	11.1 ± 4.0*
LVMD (ms)	25 (20–30)	31 (27–39)*	47 (38–57)*	62 (52–73)*
RVFWS (%)	26.6 ± 3.7	25.3 ± 3.6	21.8 ± 4.2*	14.1 ± 5.5*

Values are median (interquartile range) or mean ± SD. *Statistical significant difference in comparison to control subjects (i.e., Bonferroni-adjusted $p < 0.05$).
 DT = deceleration time; FAC = fractional area change; GLS = global longitudinal strain; LAVI = left atrial volume index; LVEDV/LVESV = left ventricular end-diastolic/end-systolic volume; LVMD = left ventricular mechanical dispersion; RV = right ventricle/ventricular; RVEDA/RVESA = right ventricular end-diastolic/end-systolic area; RVFWS = right ventricular free-wall strain, RVOT-PLAX/PSAX = right ventricular outflow tract in parasternal long-axis/short-axis view; other abbreviations as in Table 1.

(27). Images with a frame rate <50/s, foreshortened images, or images that required exclusion of >1 segment were not used for analysis. Global longitudinal strain (GLS) reflected global LV myocardial function and was defined as the average global peak strain (percentage) from the 3 apical views. GLS values were reported as absolute values. LV mechanical dispersion (LVMD) reflected heterogeneity in LV contraction and was defined as the SD of time to peak longitudinal strain (in ms) from the 18 LV segmental deformation curves.

RV deformation imaging was performed when a RV-focused apical 4-chamber view was available (28). Single-wall analysis was performed on the RV free wall. Timing of pulmonic valve closure was derived from spectral Doppler recordings in the parasternal short-axis view. RV free-wall strain (RVFWS) reflected global RV myocardial function and was defined as the average systolic peak strain (percentage) from the 3 RV free-wall segments (basal, mid, and apical). RVFWS values were reported as absolute values.

Regional deformation imaging. The 18 regional LV deformation curves were evaluated for presence of post-systolic shortening (PSS), which was defined as longitudinal myocardial shortening after aortic valve closure in any of the LV segments (Supplemental Figure 1) (29). Timing of aortic valve closure was derived from the 2-dimensional recording in the apical 3-chamber view. The degree of PSS was expressed using the post-systolic index, which was the amount

of additional negative strain after aortic valve closure as a percentage of the peak strain in 1 particular segment. In this study, PSS was considered present when the post-systolic index was $\geq 10\%$ in 1 particular segment (29). For assessment of predilection sites of PSS, we used the LV models that were automatically computed by the software package.

FOLLOW-UP DATA. Follow-up data were derived from an electronic research data platform after performing all echocardiographic measurements (21). To investigate the prognostic value of echocardiographic measurements in pre-symptomatic mutation carriers, we evaluated whether these subjects developed sustained or nonsustained VA during follow-up (i.e., at least 1 month after the date of echocardiographic examination). Because arrhythmia surveillance in these mutation carriers is usually performed by Holter recording, we only included pre-symptomatic mutation carriers in this analysis who underwent Holter recording during follow-up. Holter recording was performed upon discretion of the treating physician but is typically performed once every 2 years in these mutation carriers.

STATISTICAL ANALYSIS. Data are expressed as mean ± SD or median (interquartile range [IQR]), as appropriate. Continuous variables were compared between the 2 groups using an independent samples Student's *t*-test or Mann-Whitney U test as appropriate. Multiple groups were compared using 1-way analysis of variance or Kruskal-Wallis test as appropriate, with

FIGURE 2 Predilection Sites of PSS in Different Subgroups

Left ventricular models are presented for different subgroups. The colors depict the number of subjects with post-systolic shortening (PSS) in a particular segment. PSS was already found in pre-symptomatic mutation carriers and was most pronounced in the apical segments.

A = anterior; AS = anteroapical; I = inferior; L = lateral; S = septal; p = posterior.

adjusted p values by Bonferroni correction for multiple testing. Proportions were compared between groups using the chi-square test or Fisher's exact test as appropriate. Echocardiographic measurements that were significantly different between control subjects and pre-symptomatic mutation carriers were tested with univariable Cox regression analysis for prediction of VA among pre-symptomatic mutation carriers. The following predefined clinical variables were also tested with univariable Cox regression: age, sex, PVC count at baseline, presence of low QRS voltages at baseline, and number of leads with T-wave inversions at baseline. The single strongest echocardiographic predictor (i.e., with lowest p value in univariable analysis) was entered into multivariable analysis with other clinical variables that reached $p < 0.10$ in univariable analysis. Hazards ratios (HRs) are provided with 95% confidence intervals (CIs). Two-tailed p values of <0.05 were considered statistically significant. Statistical analyses were performed with IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp, Armonk, New York).

RESULTS

STUDY POPULATION. Baseline characteristics are presented in [Table 1](#). The final study population included 281 mutation carriers, of whom 139 (50%) were classified into the pre-symptomatic stage, 91 (32%) into the arrhythmic stage, and 51 (18%) into the structural stage. Control subjects had similar age and sex distribution as the pre-symptomatic mutation carriers.

CLINICAL CHARACTERISTICS OF PRE-SYMPTOMATIC MUTATION CARRIERS.

All pre-symptomatic mutation carriers were relatives of index patients who were screened as part of cascade family screening. The median age of pre-symptomatic mutation carriers was 33 years (IQR: 21 to 42 years), and 59 (42%) were men. By design, none of them fulfilled current diagnostic criteria for arrhythmogenic right ventricular cardiomyopathy or dilated cardiomyopathy at baseline, and none of them had an implantable cardioverter-defibrillator ([11,23](#)). All of them were classified as New York Heart Association functional class I. Nine pre-symptomatic mutation carriers (7%) were taking cardiac medication at baseline, which was eplerenone in 5 (trial medication; i-PHORECAST), an angiotensin-converting enzyme inhibitor in 3, and a beta-blocker in 1.

Holter recordings were available in 128 pre-symptomatic mutation carriers (92%). The median number of PVCs in pre-symptomatic mutation carriers was 4 (IQR: 1 to 55) per 24 h.

Electrocardiograms were available in 133 pre-symptomatic mutation carriers (96%). T-wave inversions were not assessed in 3 carriers because of a right bundle branch block. Ten pre-symptomatic mutation carriers (8%) had T-wave inversions in >1 lead. Low QRS voltages were found in 16 pre-symptomatic mutation carriers (12%).

Cardiac magnetic resonance studies were available in 78 pre-symptomatic mutation carriers (56%). LGE was present in 19 (24%) carriers and was most often seen in the LV free wall ($n = 16$; 21%), followed by hinge-point LGE ($n = 3$; 4%) and apical LGE ($n = 1$; 1%).

RV LGE was not seen in any of the pre-symptomatic mutation carriers.

ECHOCARDIOGRAPHIC EVALUATION. Conventional measurements. Echocardiographic measurements are presented in [Table 2](#). By conventional measurements, pre-symptomatic mutation carriers had similar LV and left atrial volumes and RV dimensions in comparison to control subjects. Conventional measurements for LV systolic and diastolic function and RV systolic function in pre-symptomatic mutation carriers were also similar to control subjects.

Deformation Imaging (Global). GLS was found to be lower in pre-symptomatic mutation carriers than in control subjects ($19.9 \pm 1.9\%$ vs. $21.5 \pm 1.8\%$, respectively; $p < 0.001$). GLS was even lower in the arrhythmic stage ($18.4 \pm 2.1\%$; $p < 0.001$) and in the structural stage ($11.1 \pm 4.0\%$; $p < 0.001$). Accordingly, LVMD was higher in pre-symptomatic mutation carriers than in control subjects (31 ms [IQR: 27 to 39 ms] vs. 25 ms [IQR: 20 to 30 ms], respectively; $p < 0.001$) and was even higher in the arrhythmic stage (47 ms [IQR: 38 to 57 ms]; $p < 0.001$) and in the structural stage (62 ms [IQR: 52 to 73 ms]; $p < 0.001$). RV deformation imaging was feasible in 67 pre-symptomatic mutation carriers (48%), but RVFWS was not significantly different in comparison to control subjects.

Deformation imaging (regional). Rates of PSS in different LV segments are shown in [Figure 2](#). In pre-symptomatic mutation carriers, PSS was most pronounced in the apical segments in 43 subjects (31%). Apical PSS was even more frequent in the arrhythmic stage ($n = 59$; 66%) and in the structural stage ($n = 50$; 98%). In control subjects, PSS was never seen in the apical segments. Exact rates of PSS per segment are shown in [Supplemental Table 1](#).

Characteristics of pre-symptomatic subjects with apical PSS compared with those without apical PSS are shown in [Table 3](#). Age and sex were comparable between these groups, and the conventional echocardiographic measurements were not different. Subjects with apical PSS had increased LVMD in comparison to subjects without apical PSS (40 ms [IQR: 34 to 49 ms] vs. 29 ms [IQR: 25 to 34 ms], respectively; $p < 0.001$). Subjects with apical PSS also tended to have low QRS voltages ($p = 0.064$) and LGE ($p = 0.080$) more frequently than subjects without apical PSS. However, LGE in the apical region was only seen in 1 subject with apical PSS (4%).

FOLLOW-UP OF PRE-SYMPTOMATIC MUTATION CARRIERS. Follow-up Holter recordings were available in 104 pre-symptomatic mutation carriers (75%). Pre-symptomatic subjects without follow-up Holter

TABLE 3 Characteristics of Pre-Symptomatic Subjects With and Without Apical PSS

	Apical PSS+ (n = 43)	Apical PSS- (n = 96)	p Value
Age (yrs)	33 (23–41)	33 (20–43)	0.607
Male	22 (51)	37 (39)	0.164
LVEDV (ml/m ²)	54.0 (49.4–61.4)	53.3 (45.9–59.9)	0.353
LVESV (ml/m ²)	21.4 (19.4–26.9)	20.4 (17.4–24.7)	0.284
LVEF (%)	57.5 $\pm$ 4.7	58.8 $\pm$ 4.0	0.103
RVEDA (cm ² /m ²)	9.4 (8.3–10.4)	9.5 (8.7–10.4)	0.785
RVESA (cm ² /m ²)	5.1 (4.1–5.8)	5.0 (4.4–5.8)	0.709
RV FAC (%)	46.1 $\pm$ 5.3	46.1 $\pm$ 5.7	0.951
GLS (%)	19.5 $\pm$ 2.0	20.1 $\pm$ 1.8	0.089
LVMD (ms)	40 (34–49)	29 (25–34)	<0.001*
RVFWS (%)	24.4 $\pm$ 3.9	25.8 $\pm$ 3.3	0.119
PVC count (n/24 h)	5 (2–155)	4 (1–37)	0.172
Low QRS voltages	8/40 (20)	8/93 (9)	0.064
TWI in >1 lead	5/39 (13)	5/91 (6)	0.165
Apical LGE	1/23 (4)	0/55 (0)	0.295
Any LGE	9/23 (39)	1/55 (18)	0.080

Values are as median (interquartile range), n (%), or mean $\pm$ SD. *Statistical significant difference between the 2 groups ($p < 0.05$).
PSS = post-systolic shortening; other abbreviations as in [Tables 1 and 2](#).

were typically subjects who had their baseline echocardiogram in the last 3 years of the inclusion period (30 of 35). After a median follow-up of 3.2 years (IQR: 2.1 to 5.6 years), nonsustained VA occurred in 13 subjects (13%). Sustained VA was not observed in these subjects. The median non-sustained VA cycle length was 364 ms (IQR: 274 to 430 ms), whereas the median VA duration was 4 beats (IQR: 3 to 7 beats).

Results of Cox regression analysis are shown in [Table 4](#). Of all the echocardiographic variables that were tested in univariable analysis, presence of apical PSS was the strongest predictor of VA ($p = 0.018$). Presence of apical PSS remained a significant predictor when it was entered into the multivariable analysis together with male sex (multivariable HR: 5.11; 95% CI: 1.37 to 19.08; $p = 0.015$).

Of the subjects with apical PSS at baseline with available follow-up ($n = 34$), 10 developed VA (positive predictive value: 29%; 95% CI: 21% to 40%). Of the subjects without apical PSS at baseline with available follow-up ($n = 70$), 3 developed VA (negative predictive value: 96%; 95% CI: 89% to 98%) ([Figure 3](#)). Follow-up duration was similar between pre-symptomatic subjects with and without apical PSS ($p = 0.986$). The 3 subjects without apical PSS at baseline who developed VA during follow-up were typically subjects with a long follow-up duration (characteristics shown in [Supplemental Table 2](#)).

TABLE 4 Prediction of VA in Pre-Symptomatic Mutation Carriers

	VA+ (n = 13)	VA- (n = 91)	Univariable HR (95% CI)	p Value	Multivariable HR (95% CI)	p Value
Echocardiographic variables						
GLS (%)	19.9 ± 1.6	20.0 ± 1.9	0.98 (0.67-1.47)	0.953		
LVMD (ms)	39 (30-48)	31 (26-37)	1.06 (1.00-1.11)	0.036†		
Apical PSS	10 (77)	24 (26)	4.97 (1.32-18.72)	0.018†	5.11 (1.37-19.08)	0.015†
Other clinical variables						
Age (yrs)	33 (21-39)	33 (22-42)	1.02 (0.98-1.06)	0.401		
Male	9 (69)	34 (37)	2.95 (0.91-9.65)	0.073*	3.17 (0.96-10.47)	0.059*
PVC count (n/24 h)	5 (2-105)	4 (1-39)	1.00 (0.99-1.01)	0.972		
Low QRS voltages	4/13 (31)	9/88 (10)	1.99 (0.57-7.01)	0.282		
Leads with TWI (n)	0 (0-0)	0 (0-1)	0.78 (0.28-2.21)	0.643		

Values are as mean ± SD, median (interquartile range), or n (%). The single strongest echocardiographic variable (apical PSS) was tested in multivariable Cox regression analysis with other clinical variables that reached *p < 0.10 in univariable analysis (only male sex). *p < 0.10. †p < 0.05.

CI = confidence interval; HR = hazard ratio; other abbreviations as in [Tables 1 to 3](#).

DISCUSSION

The key findings of this study are: 1) echocardiographic deformation imaging reveals global, and, in particular, regional apical mechanical alterations in pre-symptomatic PLN p.Arg14del mutation carriers; and 2) pre-symptomatic mutation carriers with abnormal apical deformation patterns appear to be at higher risk for VA. These findings imply that echocardiographic deformation characteristics may be used for early disease stage classification ([Central Illustration](#)) and have the potential to improve clinical management of pre-symptomatic mutation carriers.

EARLY DETECTION. During the systolic phase of the cardiac cycle, calcium is released from the sarcoplasmic reticulum into the cytosol, which triggers contraction of the cardiomyocyte. During diastole, calcium should be transported back into the sarcoplasmic reticulum, which is essential for rapid cardiomyocyte relaxation. In patients with the PLN p.Arg14del mutation, calcium handling is disturbed ([30](#)). It is assumed that the mutated PLN protein inhibits calcium uptake in the sarcoplasmic reticulum, which primarily leads to high concentrations of calcium in the cytosol during diastole ([5](#)).

In our study, we observed mechanical alterations in PLN p.Arg14del mutation carriers who were in early stages of disease; in particular, regional alterations were observed. PSS in the LV apex was seen in approximately one-third of pre-symptomatic mutation carriers in our cohort and in none of the control subjects. We speculate that this early diastolic phenomenon (which occurs predominantly during the isovolumetric relaxation phase) is a sign of impaired relaxation due to diastolic calcium overload in cardiomyocytes. We found that presence of apical PSS in pre-symptomatic mutation carriers was not

associated with presence of LGE in the apical region. This supports the concept that apical PSS represents an (electro-)mechanical substrate that differs from early myocardial fibrosis ([24](#)). This is also supported by previous studies that showed PSS in patients with long QT syndrome, which is characterized by prolonged action potential duration without the presence of any structural heart disease ([31,32](#)).

It remains unknown why PSS is confined to the LV apex during early stages of disease in PLN p.Arg14del mutation carriers. A previous study by Sengupta et al. ([33](#)) that used invasive measurements in healthy pigs suggested that the apical region was the last region to be repolarized in the LV and also the last one to start with diastolic lengthening. This might explain why diastolic calcium imbalance becomes first apparent in the LV apex in PLN p.Arg14del carriers. However, the normal sequence of local ventricular repolarization is still largely unknown. Therefore, this hypothesis remains to be further investigated.

DISEASE STAGE CLASSIFICATION. In this study, we subdivided pre-symptomatic mutation carriers into 2 distinct disease stages ([Central Illustration](#)). Mutation carriers with normal deformation patterns in the LV apex were found to be at low risk of developing any VA within 3 years and were therefore deemed to be in a concealed stage of disease. In contrast, mutation carriers with PSS in the apex appeared to be at higher risk of developing nonsustained VA during follow-up. Because nonsustained VA was previously found to precede occurrence of sustained VA, we consider apical PSS to be an important subclinical sign of disease ([13](#)). In contrast to regional deformation, GLS was not found to be associated with occurrence of VA in this study.

It is currently recommended to perform repeated cardiac screening in mutation carriers every 2 to 3

FIGURE 3 Prediction of VA in Pre-Symptomatic Mutation Carriers

years in case of normal cardiovascular tests (11,12). Follow-up protocols are usually intensified from late adolescence onward because of the age-related penetrance of disease (3,13,14). The results of our study suggest that deformation imaging can be used to further tailor follow-up protocols for pre-symptomatic PLN p.Arg14del mutation carriers. More specifically, because mutation carriers with apical PSS appear to be at higher risk of developing VA, follow-up frequency should be higher in these subjects compared with mutation carriers with normal deformation patterns in the LV apex. Future longitudinal studies should be conducted to determine optimal follow-up intervals in these mutation carriers. Moreover, if medical treatment becomes available for pre-symptomatic mutation carriers,

interventional studies that investigate deformation imaging as an early tool for treatment selection would also be of interest.

STUDY LIMITATIONS. For this explorative study, we considered PSS to be present when the post-systolic index was $\geq 10\%$ in a segment. However, prolonged contraction duration with less pronounced PSS might also be of clinical relevance in PLN p.Arg14del mutation carriers. Future studies should therefore aim to optimize classification of deformation patterns, for example, with machine learning algorithms.

Due to the relatively short follow-up duration, only nonsustained VA was observed in pre-symptomatic mutation carriers. The predictive value of apical PSS for life-threatening VA remains to be investigated in

CENTRAL ILLUSTRATION Disease Classification on the Basis of Myocardial Deformation Characteristics

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On the basis of presence of post-systolic shortening (PSS) in the left ventricular (LV) apex, pre-symptomatic mutation carriers were reclassified into the concealed stage (no apical PSS) or into the subclinical stage (presence of apical PSS). Subjects in the concealed stage had normal values of LV mechanical dispersion (LVMD) and global longitudinal strain (GLS), whereas subjects in the subclinical stage had increased LVMD and occasionally impaired GLS. In symptomatic subjects (arrhythmic or structural stage), myocardial deformation characteristics were even more impaired. LVEF = left ventricular ejection fraction; RVEF = right ventricular ejection fraction.

studies with longer follow-up. Furthermore, the effect of other clinical variables may be underestimated due to relatively small number of events. These variables should therefore not be neglected on the basis of our results.

Apical LGE was not observed in subjects who had apical PSS. However, absence of LGE does not exclude presence of subtle structural myocardial changes. More comprehensive cardiac magnetic resonance techniques, such as T1 mapping, would shed light on the possible underlying substrate for mechanical dysfunction.

CONCLUSIONS

Echocardiographic deformation imaging reveals global and regional mechanical alterations in PLN p.Arg14del mutation carriers before arrhythmic symptoms and overt structural disease. Pre-symptomatic mutation carriers with normal deformation patterns in the LV apex are at low risk of developing VA within 3 years, whereas mutation carriers with apical PSS appear to be at higher risk. Future studies with longer follow-up should be conducted to determine the added value of deformation imaging with regard to clinical management of pre-symptomatic mutation carriers.

AUTHOR DISCLOSURES

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: The present study demonstrates that echocardiographic deformation imaging reveals mechanical alterations in pre-symptomatic phospholamban p.Arg14del mutation carriers. By assessing deformation patterns in the LV apex, pre-symptomatic mutation carriers can be re-classified into 2 distinct disease stages: the concealed stage and the subclinical stage. The proposed disease stage classification provides opportunities to improve early management in this challenging group of at-risk individuals.

TRANSLATIONAL OUTLOOK: To elucidate whether apical PSS represents an early electromechanical substrate in pre-symptomatic mutation carriers, future studies should correlate deformation characteristics with (non-)invasive electrophysiological investigations. It would be of particular interest to study whether mutation carriers with subclinical apical PSS develop VAs originating from the apex. Moreover, more comprehensive characterization of regions with PSS with cardiovascular magnetic resonance imaging (in particular T1 mapping) would also be of interest.

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APPENDIX For an expanded Methods section and supplemental tables and figure, please see the online version of this paper.