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Multimodality imaging in the characterization and risk-stratification of cardiac disease and CRT recipients

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10

Left ventricular 2D speckle tracking echocardiography for detection of systolic dysfunction in genetic, dilated cardiomyopathies

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ABSTRACT

Background: Genetic, dilated cardiomyopathy (DCM) can be caused by a large variety of mutations. Mutation carriers are often asymptomatic until DCM is well established, presenting with heart failure, arrhythmias or sudden cardiac death. Preventive strategies can only be applied if DCM is detected early. Echocardiographic, left ventricular (LV) global longitudinal strain (GLS) is a promising tool for early diagnosis, i.e. before a decrease in LV ejection fraction (EF) has occurred. We therefore investigated the role of LV GLS as an early disease marker in genetic DCM.

Methods: Genetic DCM patients and genotyped family members were evaluated. The study population was grouped as: i) genotype-positive, phenotype-positive patients (GPFP) with a pathogenic mutation and LVEF<55%, ii) genotype-positive, phenotype-negative (GPFN) individuals with a pathogenic mutation and LVEF≥55%, and iii) genotype-negative, phenotype-negative (GNFN) individuals without a pathogenic mutation and LVEF≥55%.

Results: A total of 115 individuals (mean age 53±15 years, 51% male) were analyzed: 28 (24%) were classified as GNFN, 50 (44%) as GPFN and 37 (32%) as GPFP. Various mutations were represented: 39 (34%) titin, 14 (12%) lamin A/C, 13 (11%) sarcomeric and 21 (18%) less frequent mutations (grouped together). The mean LVEF was 58±14% for all subjects. The mean LV GLS in the GNFN group was -21.7±1.5% vs. -19.7±3.5% for the GPFN group (P=0.036). The mean LV GLS was -12.9±4.3% for the GPFP category (P<0.001 vs. GPFN and GNFN).

Conclusions: Decreased LV GLS discriminates GPFN individuals from normal controls, which may permit early institution of therapy for genetic DCM.

INTRODUCTION

Dilated cardiomyopathy (DCM) is defined as “left ventricular (LV) or biventricular systolic dysfunction and dilation that are not explained by abnormal loading conditions”.¹ There are two main etiological groups, i.e. genetic and non-genetic.¹ A broad variety of mutations underlie genetic DCM, with sarcomeric, lamin A/C (LMNA) and titin (TTN) mutations being most frequent.^{2,3} Mutation carriers often remain asymptomatic until cardiac disease is well established, and then present clinically with advanced heart failure and depressed LV ejection fraction (LVEF), life-threatening arrhythmias or sudden cardiac death.⁴ In order to institute effective preventive therapies for these serious complications, clinicians will require the ability to diagnose genetic DCM early.

Echocardiographic strain imaging is most commonly performed with 2-dimensional (2D) speckle tracking strain echocardiography and LV global longitudinal strain (GLS) is the most frequently used measure of LV systolic (dys)function. LV GLS has proven useful for diagnosing early phases of both genetic and non-genetic cardiomyopathies, as well as in risk-stratification.⁵ The utility of LV GLS in the early diagnosis of genetic DCM has not been thoroughly investigated. The purpose of the present study is therefore to investigate LV GLS as a potential marker of early LV systolic dysfunction in individuals who are mutation carriers for genetic DCM.

METHODS

Study population and data collection

Clinical and echocardiographic data from genotyped patients with DCM, as well as genotyped family members of probands, were analyzed from an ongoing clinical registry of genetic DCM. For retrospective analysis of data collected for clinical purposes and handled anonymously, the institutional review board waived the requirement for patient written informed consent. Mutation screening was clinically performed in family members with a cardiomyopathy-related gene panel. Patients gave consent for mutation screening. LV dysfunction (phenotype positive) was defined as an LVEF<55%.⁶ Mutations were considered pathogenic or likely pathogenic (genotype positive) if associated with DCM in the literature or in the local population, as described previously.^{2,7} Individuals were excluded if a mutation was considered of uncertain significance, likely benign or benign.⁷ In addition, significant valvular and coronary artery disease were exclusion criteria.

The study population was divided into three categories: i) genotype-positive, phenotype-positive (GFP) ii) genotype-positive, phenotype-negative (GPFN) and iii) genotype-negative, phenotype-negative (GNFN). Patient groups were compared in terms of LV systolic function as assessed with conventional (LVEF) and 2D speckle tracking echocardiography (LV GLS) to

investigate whether LV GLS is more sensitive than LVEF to identify the patients with subclinical DCM.

Echocardiographic data acquisition

Transthoracic echocardiography was performed in the left lateral decubitus position, using a commercially available system (E9 or VIVID 7, General Electric Vingmed Ultrasound, Milwaukee, USA) equipped with either a 3.5 MHz or a M5S transducer and with adjustment of the depth and gain settings as appropriate. M-mode, 2D and Doppler data were stored digitally after acquisition to allow for off-line analysis (EchoPac 113, General Electric Vingmed Ultrasound, Milwaukee, USA).

Chamber quantification, i.e. LV end-systolic volume (LVESV), LV end-diastolic volume (LVEDV), left atrial volume and calculation of the LVEF, was performed on 2- and 4-chamber apical views, according to contemporary guidelines.⁶ LV GLS was measured with 2D speckle tracking echocardiography and averaged from standard apical views (2-, 4- chamber, and long-axis).^{6,8}

Statistical analysis

Continuous variables are presented as means and standard deviations, and categorical data as numbers and percentages. Independent samples t-tests, as well as one-way analysis of variance (ANOVA), were employed for the comparison of continuous variables. The χ^2 and Fisher's exact tests (as appropriate) were used to compare categorical variables. Post-hoc analyses were utilized for inter-group comparisons, where appropriate. All analyses were conducted with SPSS for Windows, version 23.0 (SPSS, Armonk, NY, USA). All statistical tests were two-sided, and a P-value of <0.05 was considered statistically significant.

RESULTS

Clinical, genetic and electrocardiographic characteristics

A total of 115 persons (mean age 53±15 years, 51% male) were analyzed. Thirty-seven (32%) patients were GPF, 50 (44%) individuals were GPFN and 28 (24%) individuals were classified as GNFN. The distribution of specific, pathogenic mutations is presented in Table 1. Mutations of TTN and LMNA were the most common: 39 (34%) and 14 (12%), respectively. Baseline characteristics of individuals, according to their genotype-phenotype classification, are summarized in Table 2. Use of heart failure medication (diuretics, mineralocorticoid antagonists, angiotensin-converting enzyme (ACE)-inhibitors and beta-blockers) was significantly higher in patients who were classified as GPF, compared to GPFN and GNFN individuals (P<0.05 for both interactions). Patients in the GPF group were less frequently in sinus rhythm, and more often paced, compared to GPFN and GNFN groups (P<0.05 for both comparisons).

Conventional and 2D speckle tracking echocardiographic parameters in genotype-phenotype groups

GPFP patients had larger LV volumes and lower LVEF as compared with the other groups, but there were no differences between GNFN and GPFN groups (Table 2). The mean LV GLS for all study subjects was $-18.0 \pm 4.9\%$. Measurement of LV GLS was feasible in all 115 (100%) of patients. Interestingly, LV GLS was significantly more impaired in GPFN patients than in GNFN individuals, whereas the GPFP group showed the most impaired LV GLS (Figures 1 and 2). Therefore, in contrast to conventional echocardiographic parameters, GPFN individuals could be distinguished from GNFN controls using LV GLS.

Table 1: Distribution of mutations in genotype-positive, phenotype-negative (GPFN) and genotype-positive, phenotype-positive (GPFP) categories.

	GPFN (n=50)	GPFP (n=37)
TTN	24	15
LMNA	10	4
MYH7	0	4
MYH6	1	0
TPM1	1	1
TNNT2	0	2
MYPN	1	0
MYBPC3	2	1
ANKRD1	1	1
VCL	1	2
DSP	0	2
PLN	2	0
LAMA4	1	0
SCN5A	1	2
PKP2	1	0
PSEN1	2	2
DES	1	1
ANO5	1	0

TTN: titin, LMNA: lamin A/C, ANKRD1: cardiac ankyrin repeat protein, MYH7: β -myosin heavy chain, MYH6: α -myosin heavy chain, VCL: metavinculin, TPM1: α -tropomyosin, TNNT2: cardiac troponin T, MYBPC3: myosin-binding protein C, DSP: desmoplakin, PLN: phospholamban, LAMA4: laminin- α -4, SCN5A: sodium channel type V, PKP2: plakophilin 2, PSEN1: presenillin 1, MYPN: myopalladin, DES: desmin, ANO5: anoctamin-5.

DISCUSSION

The principal result of this study, is that LV GLS is substantially more impaired in GPFN individuals as compared to controls. Therefore, LV GLS may identify mutation carriers for genetic DCM with a normal LVEF (GPFN) at an early (subclinical) stage.

Clinical and genetic characteristics of DCM

Even though more than 50 pathogenic genes have been identified in genetic DCM, sarcomeric, LMNA and TTN mutations are the most frequent.^{2,3} In the study by Van Spaendonck et al. (which did not include TTN mutations), LMNA mutations represented 23% of the mutation-positive patients, while sarcomeric mutations accounted for another 16%.² The corresponding percentages in our study are 16% and 14%, respectively, and are comparable. Despite the fact that TTN mutations are commonly found in genetic DCM, it is still not completely clear what percentage of these mutations is truly pathogenic.¹ In a Finnish study of 145 patients with DCM, TTN mutations were identified in 20.6% of these patients.⁹ In our study, TTN mutations were present in 45% of mutation-positive (i.e. GPFN and GPPF) individuals.

Table 2: Clinical characteristics.

	GNFN (n=28)	GPFN (n=50)	GPPF (n=37)
Age (years)	52.1±13.8	50.0±14.5	56.2±16.7
Male gender, n (%)	12 (42.9)	22 (44.0)	25 (67.6)
NYHA class, n (%)			
- I	27 (96.4)	48 (96.0)	30 (81.1)
- II	1 (3.6)	1 (2.0)	3 (8.1)
- III	0 (0.0)	1 (2.0)	1 (2.7)
- IV	0 (0.0)	0 (0.0)	3 (8.1)
Medical therapy, n (%)			
- Diuretic	0 (0.0)	4 (8.0)	16 (43.2)*†
- Mineralocorticoid antagonist	0 (0.0)	3 (6.0)	12 (32.4)*†
- ACE-inhibitor	3 (10.7)	3 (6.0)	14 (37.8)†
- All antagonist	2 (7.1)	5 (10.0)	13 (35.1)
- Beta-adrenoreceptor antagonist	5 (17.9)	9 (18.0)	20 (54.1)*†
- Amiodarone	0 (0.0)	0 (0.0)	4 (10.8)
- Sotalol	0 (0.0)	1 (2.0)	3 (8.1)
- Digoxin	0 (0.0)	1 (2.0)	3 (8.1)
- Anticoagulation	1 (3.6)	4 (8.0)	15 (40.5)*†
Heart rhythm, n (%)			
- Sinus rhythm	26 (92.9)	49 (98.0)	22 (59.5)†
- Paced rhythm	0 (0.0)	1 (2.0)	14 (37.8)*†
- Atrial fibrillation	2 (7.1)	0 (0.0)	1 (2.7)
AV block, n (%)			
- 1 st degree	0 (0.0)	2 (4.0)	3 (8.1)
- 2 nd degree	0 (0.0)	2 (4.0)	0 (0.0)
- 3 rd degree	0 (0.0)	0 (0.0)	1 (2.7)
LA volume (ml)	34.7±13.4	36.8±12.5	51.0±26.2*†
LVEF (%)	66.8±5.7	64.3±6.7	42.2±11.0*†
LVEDV (ml)	77.9±23.2	80.2±25.0	106.3±51.3*†
LVESV (ml)	26.2±9.3	29.3±12.4	65.7±48.8*†

Values are mean ± standard deviation. All: angiotensin II receptor antagonist, ACE: angiotensin converting enzyme inhibitor, AV: atrioventricular, GNFN: genotype-negative phenotype-negative, GPFN: genotype-positive phenotype-negative, GPPF: genotype-positive phenotype-positive, LA: left atrial, LVEF: left ventricular ejection fraction, LVEDV: left ventricular end-diastolic volume, LVESV: left ventricular end-systolic volume, NYHA: New York Heart Association, *P<0.05 vs. GNFN, †P<0.05 vs. GPPF.

The identification of a pathogenic mutation does not necessarily imply the presence of clinically-relevant cardiac disease, nor does it aid in risk-stratification. Alternative strategies are therefore required for identifying cardiac involvement and predicting the risk of complications.

Conventional echocardiographic parameters in genetic DCM

Lakdawala et al. compared 21 patients with overt, genetic DCM, 12 asymptomatic genotyped family members with disease-causing mutations (subclinical DCM) and 29 normal controls.¹⁰

“Overt” genetic DCM was defined as an LVEF <55% and/or LV enlargement (according to published reference values).¹⁰ The LV end-diastolic diameter (LVEDD) was not found to be significantly different between the subclinical group (4.3 ± 0.7 cm) and the controls (4.3 ± 0.6 cm) ($P=0.65$).¹⁰ Neither the LV end-systolic diameter (LVESD) in individuals with “subclinical

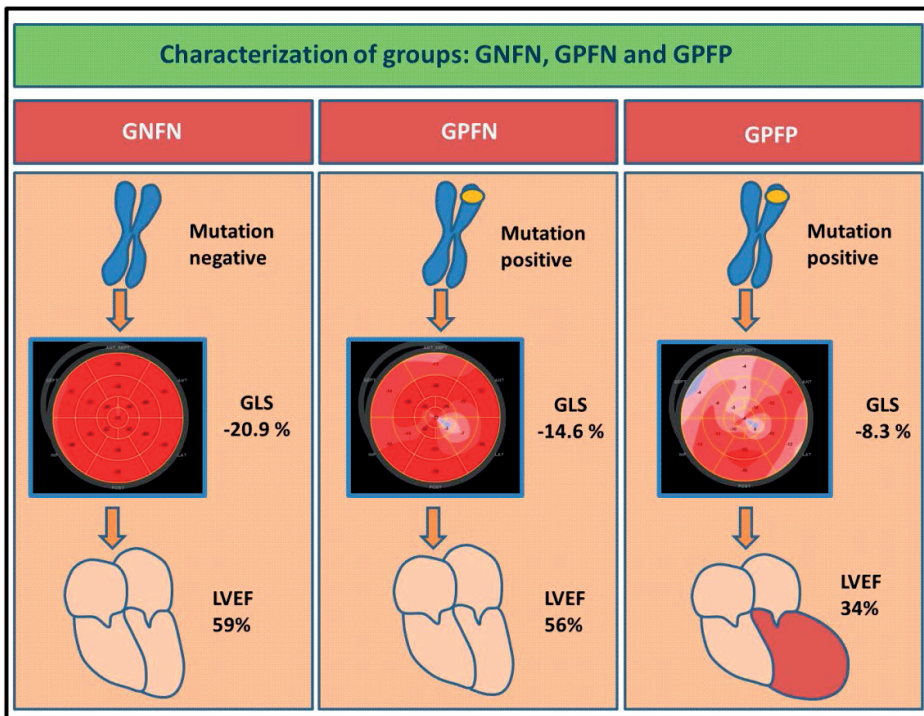


Figure 1: Characterization of groups: GNFN, GPFN, GPFP. The genotype status, global longitudinal, left ventricular strain (GLS) and left ventricular ejection fraction (LVEF) of three different individuals are shown to illustrate the following groups: genotype-negative, phenotype-negative (GNFN), genotype-positive, phenotype-negative (GPFN) and genotype-positive, phenotype-positive (GPFP). On the left-sided panel, a GNFN individual is shown, without a genetic mutation, with a normal GLS of -20.9% and a normal LVEF of 59%. The patient in the middle panel has a titin mutation, an impaired GLS of -14.6% and a normal LVEF of 56%, and is therefore GPFN. In the right-hand panel, a GPFP patient is shown, with a lamin A/C mutation, an impaired GLS of -8.3% and a depressed LVEF of 34%. Despite a normal LVEF of $\geq 55\%$, reduced GLS distinguishes the individual in the middle panel from a normal control (left-sided panel).

DCM" (3.1 ± 0.6 cm) and controls (2.8 ± 0.5 cm) ($P=0.09$) nor the LVEF ($59 \pm 3\%$ and $62 \pm 5\%$, respectively; $P=0.07$) could discriminate individuals with "subclinical DCM" from controls.¹⁰ In a study of 674 first-degree relatives of patients with familial DCM, only 50 (7%) demonstrated evidence for cardiac involvement by either LV dilatation or reduced LVEF on echocardiographic screening.¹¹ Baig et al. interrogated a cohort of 225 family members of patients with familial DCM on echocardiography, and also found only a small percentage (3%) with reduced LVEF.¹² Therefore, conventional echocardiographic parameters (LVEDD, LVESD, LVEF) are unable to reliably distinguish GPFN patients from GNFN controls. Our data are in agreement with these findings, and argue in favor of exploring novel echocardiographic parameters with which to diagnose genetic DCM early.

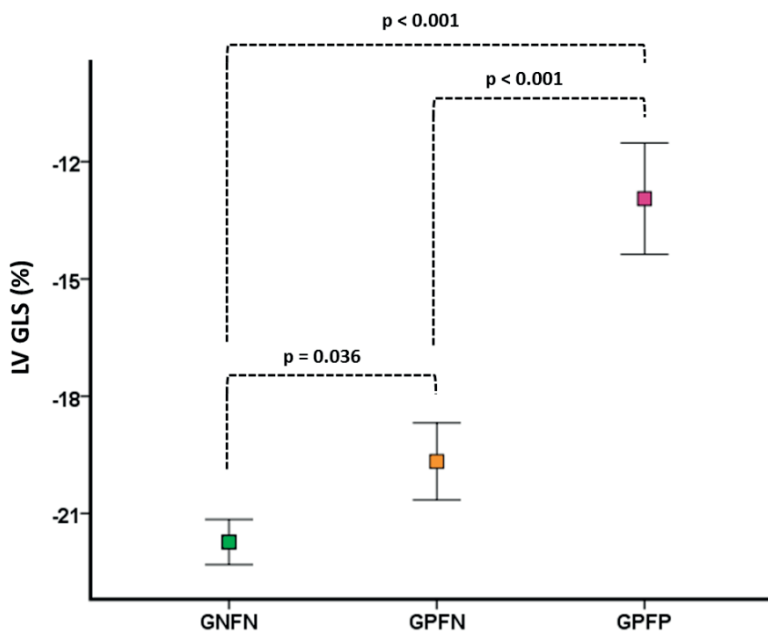


Figure 2: Left ventricular strain, according to genotype-phenotype group. Mean left ventricular global longitudinal strain (LV GLS), stratified according to the following groups: genotype-negative, phenotype-negative (GNFN), genotype-positive, phenotype-negative (GPFN) and genotype-positive, phenotype-positive (GPFP). Color-coded squares indicate mean values of GLS, while horizontal bars represent 95% confidence intervals.

The role of 2D speckle tracking strain echocardiography to assess LV longitudinal strain in genetic DCM

Early diagnosis is integral to the process of risk-stratification in genetic DCM, since affected individuals are often asymptomatic until established heart failure, life-threatening arrhythmias or sudden cardiac death occurs.⁴ Effective, preventive strategies can only be implemented if genetic DCM can be reliably diagnosed at an early stage of the disease process.

Structural signs of early genetic DCM have been described with cardiac magnetic resonance (CMR) imaging. In a cohort of 41 LMNA mutation carriers, late gadolinium enhancement (LGE)

on CMR was visualized in the basal septum only in individuals who experienced ventricular arrhythmias, while LVEF was similar in those with and without ventricular arrhythmias ($56 \pm 13\%$ vs. $53 \pm 14\%$; $P=0.55$).¹³ In the same study, there were also no significant differences in LVEDD (51 ± 9 mm vs. 54 ± 9 mm; $P=0.34$) or LVESD (36 ± 10 mm vs. 39 ± 9 mm; $P=0.29$) between subjects with and without ventricular arrhythmias.¹³ Basal, septal LGE therefore appears to be a sign of early disease, which could not be diagnosed with conventional echocardiographic parameters.¹³ The myocardial extracellular volume fraction, determined by CMR imaging, was increased in 19 LMNA mutation carriers ($28.0 \pm 3.2\%$) compared to controls ($22.7 \pm 3.0\%$) ($P<0.001$).¹⁴ Even though the indexed LVEDV was slightly higher in mutation carriers than in controls (87 ± 20 ml/m² vs. 76 ± 12 ml/m², respectively; $P<0.05$), LVEF was normal in both groups.¹⁴ Although arrhythmia risk was not specifically addressed, 3 LMNA mutation carriers (16%) had sustained ventricular tachycardia on ECG monitoring, while another 3 (16%) manifested non-sustained ventricular tachycardias.¹⁴ Reverse LV remodeling has been documented with early therapy in selected subgroups of genetic DCM, e.g. beta-blockers and systemic steroids in patients with Duchenne and Becker muscular dystrophy, probably reflecting an effect on myocardial fibrosis.^{15,16}

Impaired LV GLS (partially) reflects myocardial fibrosis in patients with heart failure, and is more accurate in this regard than LVEF or other deformation parameters (e.g. LV global circumferential strain).¹⁷ The functional impairment seen in early genetic DCM (impaired LV GLS) therefore most likely follows structural abnormalities, such as myocardial fibrosis, although the contribution of a purely functional (e.g. sarcomeric dysfunction) component cannot be excluded. In the study by Lakdawala et al., LV GLS was significantly decreased in individuals with “subclinical” genetic DCM, compared to normal controls, and LV GLS was lower in patients with “overt” genetic DCM, compared to the “subclinical” group ($P<0.001$ for all interactions).¹⁰ Of note, all mutations were sarcomeric, and >90% of individuals were female.¹⁰ In contrast, TTN, LMNA, sarcomeric and other mutations were represented in our study, with about half of the individuals being male. This is in agreement with published mutation and gender distributions for genetic DCM.^{2,9} The key finding of our study is that LV GLS is substantially lower in GPFN individuals than in normal controls (GNFN) in a representative cohort. Therapy which is instituted early, has already been proven to delay the onset or slow the progression of systolic dysfunction in some types of genetic DCM, e.g. systemic steroids and ACE-inhibitors in patients with Duchenne muscular dystrophy.^{18,19}

It is therefore clear that GPFN individuals who cannot be reliably identified by conventional echocardiographic parameters, demonstrate structural and functional cardiac abnormalities on different imaging modalities (CMR and 2D speckle tracking strain echocardiography). The results of the present study support the validity of identifying GPFN persons with 2D speckle tracking strain echocardiography, using LV GLS.

The true value of early diagnosis in patients with genetic DCM can only be addressed by prospective studies, investigating both the predictive value of imaging markers (e.g. LV GLS) for the development of a positive phenotype (decreased LVEF, dilated LV, arrhythmias, heart

failure, sudden cardiac death), as well as the efficacy of early initiation of preventive measures, e.g. standard heart failure therapy with ACE-inhibitors or primary prevention and implantable cardioverter defibrillator implantation.

Study limitations

This was a retrospective, single-center study. Nonetheless, all the major groups of mutations associated with genetic DCM are well represented. Subgroup analyses were not conducted on different mutation groups, due to the limited study population. In addition, the association between LV GLS and hard endpoints, such as arrhythmic events, was not evaluated since this was beyond the scope of the present study and the follow-up was relatively short (median follow-up was 4 months (interquartile range (IQR) 1-25)) to observe a significant number of events that can lead to clinically meaningful conclusions. Likewise, LV diastolic function was not evaluated. Measurements of LV GLS are not vendor-independent, and the threshold for reduced LV GLS may vary among different echocardiographic vendors, although the amount of variation is acceptable for clinical use.⁸ Finally, CMR data were not systematically available in this population to perform tissue characterization analysis.

CONCLUSIONS

Mutation carriers in genetic DCM (GPFN) often remain asymptomatic until presenting with advanced cardiac disease. Decreased LV GLS can help to discriminate GPFN individuals from normal controls (GNFN). This may allow early disease detection in genetic DCM. Larger, prospective studies are required to confirm these findings.

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