Feasibility of real-time cardiac MRI in mice using tiny golden angle radial sparse

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Cardiovascular magnetic resonance imaging has proven valuable for the assessment of structural and functional cardiac abnormalities. Even although it is an established imaging method in small animals, the long acquisition times of gated or self-gated techniques still limit its widespread application. In this study, the application of tiny golden angle radial sparse MRI (tyGRASP) for real-time cardiac imaging was tested in 12 constitutive nexilin (Nexn) knock-out (KO) mice, both heterozygous (Het, \( N = 6 \)) and wild-type (WT, \( N = 6 \)), and the resulting functional parameters were compared with a well-established self-gating approach. Real-time images were reconstructed for different temporal resolutions of between 16.8 and 79.8 ms per image. The suggested approach was additionally tested for dobutamine stress and qualitative first-pass perfusion imaging. Measurements were repeated twice within 2 weeks for reproducibility assessment. In direct comparison with the high-quality, self-gated technique, the real-time approach did not show any significant differences in global function parameters for acquisition times below 50 ms (rest) and 31.5 ms (stress). Compared with WT, the end-diastolic volume (EDV) and end-systolic volume (ESV) were markedly higher (\( P < 0.05 \)) and the ejection fraction (EF) was significantly lower in the Het Nexn-KO mice at rest (\( P < 0.001 \)). For the stress investigation, a clear decrease of EDV and ESV, and an increase in EF, but maintained stroke volume, could be observed in both groups. Combined with ECG-triggering, tyGRASP provided first-pass perfusion data with a temporal resolution of one image per heartbeat, allowing the quantitative assessment of upslope curves in the blood-pool and myocardium. Excellent inter-study reproducibility was achieved in all the functional parameters. The tyGRASP is a valuable real-time MRI technique for mice, which significantly reduces the scan time in preclinical cardiac functional imaging, providing sufficient image quality for deriving accurate functional parameters, and has the potential to investigate real-time and beat-to-beat changes.

Abbreviations used: BPM, beats per minute; b-SSFP, balanced steady-state free precession; CO, cardiac output; CoV, coefficient of variation; CPM, cycles per minute; CS, compressed sensing; DCM, dilated cardiomyopathy; EDV, end-diastolic volume; EF, ejection fraction; ESV, end-systolic volume; GA, golden angle; GRAPPA, generalized autocalibrating partially parallel acquisitions; HR, heart rate; Het, heterozygous; IG, self-gated sequence; KO, knock-out; LV, left ventricle; LVMED, left ventricle mass at end diastole; LVMES, left ventricle mass at end systole; Nexn, nexilin; ROI, region of interest; RT, real-time; SAX, short axes orientations; SD, standard deviation; semi-2CH, semi-two-chamber; semi-4CH, semi-four-chamber; SI, signal intensity; SV, stroke volume; tyGA, tiny golden angle; tyGRASP, tiny golden angle radial sparse; WT, wild-type.

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1 | INTRODUCTION

Cardiovascular magnetic resonance (CMR) imaging has proven to be an accurate technique in the detection and characterization of structural and functional cardiac abnormalities over the past 25 years. In particular, its versatility, accuracy and high reproducibility has made CMR the noninvasive reference modality for deriving global and regional myocardial function, quantification of fibrotic myocardium, and more advanced tissue characterization in clinical and preclinical diagnosis.

In preclinical research, small rodent animal models of human cardiovascular disease are frequently used to investigate the basic underlying mechanism of normal and abnormal cardiac function and development, and for monitoring the disease progression under therapy. However, the small size of the mouse heart (5-6 mm left ventricle [LV] diameter, ~ 0.2 g of heart weight), high heart rates (~ 250-600 beats per minute [bpm]), high respiratory rates (~ 60-160 cycles per minute [cpm]), and fast systemic blood circulation time (4-5 heartbeats) impose substantial challenges for functional assessment by MRI. Rapid data acquisition strategies for functional MRI with high temporal resolution, while preserving adequate spatial resolution and sufficient volumetric coverage, are required.

Where in its beginning small rodent MRI was based on ECG-triggered acquisition techniques, over recent years, self-gating techniques have been introduced, enabling high-quality cardiac MRI with high reproducibility. In combination with ultrashort echo time (UTE) techniques, improved image quality was reported by susceptibility, motion and flow artifact reduction. However, the acquisition times in the minute range for a single slice still limit its application, eg for pharmacological stress or first-pass perfusion imaging. To reduce acquisition times, the application of real-time (RT) methods not demanding any gating (real time) for the rapid and continuous acquisition of image datasets, such as parallel imaging, k-t acceleration methods, and compressed sensing (CS), have been suggested and initially evaluated in mice. Radial trajectories have shown favorable properties for RT imaging by their intrinsic low motion artifact level. Further, due to the continuous recording of all spatial frequencies with every single spoke, undersampling results in almost incoherent artifacts, often showing no noticeable effect on the reconstructed images. The concept of golden angle (GA) angular spacing enabled data acquisition with optimal k-space coverage that was almost independent of the number of projections (Fibonacci sequence) and ensured incoherent undersampling artifacts. Its extension to tiny golden angle (tyGA) enabled the translation of the GA principle to higher field strength and provided even larger flexibility in the selection of the number of projections used for reconstruction of a single frame (generalized Fibonacci sequence). The combination with sparse sense reconstruction (tyGRASP) has proven beneficial artifact properties for RT cardiac imaging.

It was the objective of this study to investigate the feasibility of applying tyGRASP for the quantification of the global left ventricular function from continuously acquired nongated RT data and for the qualitative assessment of first-pass perfusion with single heartbeat temporal resolution in mice. The technique was tested in a group of constitutive nexilin (Nexn) heterozygous (Het) and wild-type (WT) knock-out (KO) mice as a heart failure model. Mutations in the nexilin gene were found to cause dilated cardiomyopathy (DCM) in human patients, and functional analyses in zebrafish and mouse models demonstrated a key role of Nexn DCM-induced severe heart failure.

2 | MATERIALS AND METHODS

2.1 | Animals and ethical considerations

Twelve constitutive Het (N = 6; three male and three female) and WT (N = 6; three male and three female) Nexn KO mice were included in this study. Before the start of the study, the animals were housed in a temperature-controlled environment for at least 1 week. Facility rooms were maintained at a constant temperature (23°C) and humidity (50% relative humidity) on a 12-hour light/dark cycle. Access to food and tap water was ad libitum.

Animal experiments were approved by the regional board of Tübingen and conducted according to German law for the welfare of animals and regulations for the care and use of laboratory animals. All institutional and national guidelines for the care and use of laboratory animals were followed and approved by the appropriate institutional committees.

2.2 | CMR image acquisition

All experiments were performed on an 11.7 T dedicated small animal MRI system (BioSpec 117/16, Bruker Biospin, Ettlingen, Germany) equipped with a high-performance gradient system (B-GAS HP, Bruker Biospin), providing a maximal gradient strength of 760 mT/m with a maximal slew-
rate of 6840 T/m/s. Excitation was performed with a 72 mm quadrature transmit/receive coil and all data were acquired with a dedicated four-
element thorax coil (RAPID Biomedical, Rimpar, Germany). Slice planning was performed as suggested earlier\textsuperscript{10} ensuring high reproducibility of the image geometry for subsequent examinations. In short, the imaging protocol comprised a multi-slab survey acquisition in axial, coronal and sagittal orientation, followed by two long axis CINE scans in semi-two-chamber (semi-2CH) and semi-four-chamber (semi-4CH) geometry. The semi-2/4CH images were used to plan the subsequent stack of short axes orientations (SAX) acquired subsequently. The number of short axis slices was adopted to ensure full coverage of the LV in end diastole.

### 2.3 | Retrospectively self-gated functional MRI

Reference functional data were acquired by applying a conventional Cartesian self-gated sequence (IntraGate, ParaVision 6.0.1, Bruker Biospin)\textsuperscript{10}. Data were acquired with a high-resolution (IG-HR, echo/repetition time [TE/TR] = 1.04/5.62 ms, flip angle [\(\alpha\)] = 15\(^\circ\), matrix size = 256 x 256, spatial resolution = 0.117\(^2\) mm\(^2\), slice thickness [\(s_D\)] = 1 mm, bandwidth [\(bw\)] = 125 kHz, 20 frames per cardiac cycle, oversampling = 200, acquisition time [\(T_{ACQ}\)] = 2 minutes 37 seconds per slice) and a low-resolution (IG-LR, TE/TR = 0.85/5 ms, \(\alpha = 15^\circ\), matrix size = 150 x 150, spatial resolution = 0.2\(^2\) mm\(^2\), \(s_D = 1\) mm, \(bw = 250\) kHz, 20 frames per cardiac cycle, oversampling = 200, \(T_{ACQ} = 1\) minute 27 seconds per slice) protocol.

### 2.4 | RT nongated functional MRI

A tiny GA trajectory (Figure 1A) as suggested by Wundrak et al\textsuperscript{25} was implemented on the small animal system (ParaVision 6.0.1, Bruker Biospin). For RT imaging, the spatial resolution was chosen as similar to the low-resolution reference data acquisition, with MR acquisition parameters of TE/TR = 0.86/2.1 ms, \(\alpha = 20^\circ\), matrix size = 150 x 150, spatial resolution = 0.2\(^2\) mm\(^2\), \(s_D = 1\) mm, \(bw = 250\) kHz and \(T_{ACQ} = 1.98\) seconds per slice.

**FIGURE 1** Nongated RT acquisition: (A) radial spokes were continuously acquired with a tiny golden angle angular increment of \(\varphi_T = 23.62814^\circ\). (B) For the RT functional data, images were reconstructed from \(G_5^5 = 8\) (RT1), \(G_5^2 = 15\) (RT2), \(G_5^2 = 23\) (RT3) and \(G_5^5 = 38\) (RT4) spokes, yielding temporal resolutions of \(\Delta t = 16.8, 31.5, 48.3\) and 79.8 ms. In all cases, a sliding window reconstruction was performed with offsets and a temporal increment of 8.4 ms (four spokes) to enable comparison of the resulting image data at the same time point of the continuous data acquisition.
For ensuring short TE, excitation was performed with an asymmetric Sinc Gauss-shaped excitation pulse. During $T_{\text{ACQ}}$, 942 radial spokes were continuously acquired with a tyGA angular increment of $\phi = 23.62814^\circ$. According to the generalized Fibonacci sequence $G_1^N = 1; G_2^N = N; G_n^N = G_{n-1}^N + G_{n-2}^N$, for any subset of acquisitions containing $G_7^2 = 8$ (RT1), $G_7^3 = 15$ (RT2), $G_7^4 = 23$ (RT3) and $G_7^5 = 38$ (RT4) radial spokes, an almost homogeneous coverage of k-space was ensured with resulting temporal resolutions of $\Delta t = 16.8, 31.5, 48.3$ and 79.8 ms (Figure 1A).24

Prior to each tyGRASP acquisition, the actual trajectory was mapped independently for all gradient directions as suggested by Zhang et al.32 and applied for gradient distortion consideration during reconstruction.

2.5 | First-pass perfusion

For first-pass perfusion assessment, the tyGRASP sequence was combined with block-wise cardiac synchronization and saturation-recovery contrast (Figure 2). During each cardiac cycle, a single block of $G_7^4 = 15$ projections with an acquisition duration of $\Delta t = 31.5$ ms was acquired. The tyGA angular increment was maintained over the whole acquisition thus allowing the combination of data acquired during subsequent cardiac cycle for improving k-space coverage with compromised temporal fidelity.

For suppression of the background signal, a nonselective saturation pulse was additionally applied prior to each acquisition block with a saturation recovery time ($T_{\text{SAT}}$) of 60 ms. The trigger delay was chosen such that the acquisition was performed during end diastole to further minimize motion artifacts.

2.6 | Data reconstruction

All gated functional MR data were reconstructed by the reconstruction software provided by the vendor (IntraGate, Bruker, Ettlingen, Germany).

The tyGRASP data were reconstructed with an in-house developed reconstruction framework implemented in Matlab (The MathWorks, Natick, MA), applying a compressed sense reconstruction with total variation sparsity operator along the spatial and temporal domain according to

$$\text{argmin} \left\{ \left\| F \cdot S \cdot x - b \right\|^2_2 + \lambda_s \left\| \nabla_s x \right\|^1_1 + \lambda_t \left\| \nabla_t x \right\|^1_1 \right\},$$

with $F$ being the nonuniform Fourier transformation between Cartesian $x-t$ and radial $k-t$ space and $S$ containing the coil sensitivity maps for the receive coils. The $l_2$-norm enforces data consistency between $k-t$ space samples $b$ and the reconstructed image sequence $x$ in $x-t$ space. $\nabla_s$ and $\nabla_t$ denote the gradient operator along the spatial and temporal dimension, favoring piecewise linear solutions. In all cases 50 iterations were used and the strength of the spatial ($\lambda_s$) and temporal ($\lambda_t$) regularization was fixed to $\lambda_s = 0.05$ and $\lambda_t = 0.005$.

Coil sensitivity maps ($S_c$) were derived from the temporal average image ($I_c$) independently reconstructed for each coil ($c$) according to

$$S_c = \sqrt{\sum_{t} \frac{I_c}{I_c}}.$$

During reconstruction, the mapped real trajectory and the resulting k-space density of sampling points were fully considered.
For the RT functional data, images were reconstructed from $G_7^3 = 8$ (RT1), $G_7^4 = 15$ (RT2), $G_7^5 = 23$ (RT3) and $G_7^6 = 38$ (RT4) spokes, yielding temporal resolutions of $\Delta t = 16.8$, 31.5, 48.3 and 79.8 ms (Figure 1B). In all cases a sliding window reconstruction was performed with a temporal increment of 8.4 ms (four spokes) to enable comparison of the resulting image data at the same time point of the continuous data acquisition. The reference time point of each image was defined as the acquisition time of the central spoke of the respective data package used for reconstruction.

For first-pass perfusion, data were reconstructed from single-beat (15 spokes) and triple-beat (3 x 15 spokes) data by applying a sliding window reconstruction with single-beat (15 spokes) temporal increment (Figure 2).

2.7 | In vivo measurements

All mice were anesthetized with isoflurane (5% for induction, 1%-1.5% for maintenance, to maintain the respiratory frequency between 60-80 respiratory cycles per minute) in medical air (0.1 L/min). A water blanket was used for maintaining the temperature of the animal. During scanning, the respiratory rate (balloon pressure sensor) and the temperature of the mice (rectal temperature probe) were continuously monitored.

2.8 | Assessment of LV function

The self-gated (IG-HR, IG-LR) and nongated RT imaging protocols were performed in 12 constitutive Nexn KO mice, Het ($N = 6$) and WT ($N = 6$). For comparison, data of a short axis stack completely covering the LV were acquired. End-diastolic volume (EDV), end-systolic volume (ESV), stroke volume (SV), ejection fraction (EF), LV mass at end diastole (LVMED) and end systole (LVMES) were quantified with Segment33 (Medviso AB, Lund, Sweden) and compared between the nongated RT (RT1–RT4) and self-gated (IG-HR, IG-LR) acquisition protocols. For the continuous RT data, the end-diastolic and end-systolic phase was identified manually according to the maximal and minimal LV diameter apparent over the image series. The segmentation of the LV myocardium and cavity were performed manually by an experienced physician.

2.9 | Dobutamine stress MRI

For assessment of the performance of the RT techniques under pharmacological stress, the Nexn mice were additionally scanned after intraperitoneal (ip) injection of 1.5 $\mu$g/g body weight dobutamine.34,35 To ensure almost constant conditions between the non- and self-gated protocols, only IG-LR and RT data were acquired thus ensuring data acquisition of both short-axis stacks within less than 15 minutes. Data acquisition was started 7 minutes after ip administration of dobutamine. IG-LR and RT protocols were acquired in random order. LV functional parameters (see above) were compared between RT and IG-LR acquisition protocols.

2.10 | First-pass perfusion MRI

Two weeks after dobutamine stress imaging, the Nexn mice were additionally scanned for assessment of the feasibility of first-pass perfusion imaging with an ECG-triggered saturation recovery tyGRASP technique (Figure 2).

Data acquisition was performed in a midventricular slice. ECG was recorded from two electrodes placed at the right front and left hind paws. A homemade catheter (30.5 G needle, 200 $\mu$m hose), preloaded with heparinized saline (0.5 ml heparin [1000 USP units/ml] in 10 ml of sterile saline), was introduced into the tail vein. Scanning was performed for ~45 seconds. About 3 seconds after the start of the data acquisition, a bolus of 0.1 mmol/kg Gd-DTPA in 0.9% NaCl was injected (~35 $\mu$l bolus on average) with an infusion pump (AL-1000, WPI, flow rate: 2 ml/min).

After reconstruction of the first-pass perfusion images from single- and triple-beat datasets, regions of interest (ROIs) were placed covering the entire LV lumen and the entire LV ventricle. To avoid partial volume effects the border regions were excluded from the respective ROIs. Signal intensity (SI)-time curves and the upslope $U = (S_{N-peak}-S_{N-baseline})$/heartbeats of the normalized signal intensities, $S_{N} = (SI-S_{min})/(S_{max}-S_{min})$, were calculated.

2.11 | Inter-study reproducibility of RT imaging

For assessment of the reproducibility of the nongated approach, the LV functional imaging (RT) was repeated after 2 weeks in the Nexn WT mice and the LV functional parameters (EDV, ESV, SV, EF, LVMED and LVMES) quantified for the two most promising protocols (RT1–RT2) identified in the first experiments.
2.12 | Data quality analysis

The signal-to-noise ratio (SNR) in the myocardium and the LV blood, the contrast-to-noise ratio (CNR) between myocardium and LV blood, and the sharpness over the LV, were quantified in all animals for the relevant investigated protocols (IG-HR/IG-LR, RT1 - RT2) in a midventricular slice during end diastole. To avoid any severe impact of arising streak artifacts and coil sensitivities upon the SNR and CNR values, the calculation was performed according to $SNR = \frac{\mu}{\sigma}$ and $CNR = \frac{\mu_b - \mu_m}{\sigma_b + \sigma_m}$, with $\mu_i$ and $\sigma_i$ being the mean and standard deviation over the LV cavity ($\mu_b, \sigma_b$) and the myocardium ($\mu_m, \sigma_m$). Image sharpness was derived from the mean value of a normalized Sobel-filtered fixed ROI encompassing the heart.

2.13 | Statistical analysis

All image analyses were performed blinded. Continuous data were presented as mean ± standard deviation (SD). Non- and self-gated sequences were compared by applying Tukey HSD with repeated measures ANOVA. Differences between WT and Nexn KO mice (functional parameter; upslope of the myocardial contrast uptake) were assessed by an unpaired Mann–Whitney U test. Image quality measures (SNR, CNR and image sharpness) were analyzed by applying a one-way ANOVA test. The inter-study reproducibility was assessed using intra-class correlation coefficient (ICC), coefficient of variation (CoV) and Bland–Altman analysis. Differences were considered statistically significant with P-values of <0.05. For all the tests, *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001.

3 | RESULTS

Data acquisition could be completed in all animals. In all cases, the respiratory rate could be maintained between 60 and 80 cycles per minute and changes in the recorded temperature were below +/- 0.5°C.

3.1 | Gated and nongated LV function at rest

An example of the resulting image quality for the different imaging protocols is shown for a midventricular slice for one case of Nexn WT and Het mice in Figure 3 and the supporting information (supplemental material 1). An example of all the RT protocols from apical to basal slice are also shown in the supporting information (supplemental material 2). Even although the noncardiac structures are not clearly visible in the RT images,
the LV can be well appreciated and quantitative assessment of the functional parameters was possible for all investigated RT protocols. Comparison between the RT data ($G^3_7 = 8$ [RT1], $G^3_7 = 15$ [RT2], $G^3_7 = 23$ [RT3]) and the self-gating protocols did not reveal any statistically significant differences for all investigated parameters in the WT group (Table 1). However, for $G^3_6 = 38$ (RT4), a significant reduction of the EDV, SV and EF, and increased ESV, were observed ($P < 0.0001$). No statistically significant differences for LVM at end diastole ($P = 0.22$) and end systole ($P = 0.39$) were observed.

### 3.2 | Gated and nongated LV function at pharmacological stress

For the stress investigation, after dobutamine injection, a clear decrease of the LV EDV and ESV, an increase in EF, and maintained SV, were observed in the WT mice (Table 2). No statistically significant differences were observed between the functional parameters derived from the IG-LR and RT1/2. However, an increased ESV and reduced EF were observed in RT3 ($P < 0.05$), and more obviously in RT4, which even showed a significant reduction in the SV ($P < 0.0001$).

### 3.3 | Comparison of Nexn WT and Het mice

The heart rates increased in both groups after dobutamine injection (WT, 424 ± 45 vs. 366 ± 28, Het, $P = 0.06$, rest; WT, 475 ± 25 vs. 429 ± 31, Het, $P = 0.03$, stress). Compared with WT (Figure 4), the EDV and ESV were markedly higher in the Het Nexn-KO mice, resulting in a relatively lower EF at rest ($P < 0.01$). No statistically significant difference for LVM at end diastole and end systole was observed between the two groups ($P > 0.05$). For the functional parameters at stress, no significant difference was observed between these two groups ($P > 0.05$).

### TABLE 1
Comparison of LV functional parameters in Nexn-WT mice at rest

|       | IG-HR | IG-LR | RT1    | RT2    | RT3    | RT4    |
|-------|-------|-------|--------|--------|--------|--------|
| EDV   | 56.01 ± 5.55 | 55.45 ± 5.73 | 57.12 ± 7.26 | 56.47 ± 6.10 | 55.77 ± 6.60 | 48.86 ± 5.49 **** |
| ESV   | 14.09 ± 2.33 | 13.82 ± 2.69 | 13.24 ± 2.49 | 13.38 ± 2.48 | 14.07 ± 2.23 | 22.49 ± 4.05 **** |
| SV    | 41.92 ± 3.52 | 41.47 ± 4.06 | 43.88 ± 4.19 | 43.09 ± 3.85 | 41.70 ± 4.71 | 26.37 ± 5.79 **** |
| EF    | 75.13 ± 2.45 | 75.10 ± 2.88 | 76.93 ± 2.48 | 76.46 ± 2.33 | 74.84 ± 2.12 | 53.72 ± 8.50 **** |
| LVMES | 102.50 ± 15.42 | 102.83 ± 15.14 | 103.52 ± 14.08 | 103.35 ± 16.72 | 105.01 ± 17.15 | 102.73 ± 16.11 |
| LVMED | 100.33 ± 15.13 | 99.33 ± 14.79 | 101.38 ± 16.44 | 99.31 ± 16.96 | 102.51 ± 17.31 | 101.53 ± 16.54 |

Abbreviations: EDV, end-diastolic volume; EF, ejection fraction; ESV, end-systolic volume; IG-HR/LR, intra-gate high/low resolution protocols; LVMED/ES, end-diastolic/systolic left ventricular mass; RT1-4, RT protocol with $G^3_7 = 8$, $\Delta t = 16.8$ ms; $G^3_7 = 15$, $\Delta t = 31.5$ ms; $G^3_7 = 23$, $\Delta t = 48.3$ ms; $G^3_7 = 38$, $\Delta t = 79.8$ ms; SV, stroke volume.

Results are expressed as mean ± standard deviation. Compare with IG-HR protocol, significant reduction of the EDV, SV, EF and increased ESV were observed in RT4. ****$P < 0.0001$.

### TABLE 2
Comparison of LV functional parameters in Nexn-WT mice at dobutamine stress

|       | IG-LR | RT1    | RT2    | RT3    | RT4    |
|-------|-------|--------|--------|--------|--------|
| EDV   | 49.48 ± 5.53 | 50.95 ± 5.45 | 50.74 ± 5.98 | 50.68 ± 5.45 | 48.63 ± 6.12 |
| ESV   | 6.95 ± 0.67 | 6.68 ± 0.95 | 7.26 ± 0.77 | 10.57 ± 1.28 **** | 25.12 ± 4.15 **** |
| SV    | 42.53 ± 5.43 | 44.26 ± 4.77 | 43.48 ± 5.69 | 40.11 ± 4.86 | 23.50 ± 5.54 **** |
| EF    | 85.86 ± 1.72 | 86.88 ± 1.20 | 85.59 ± 1.67 | 79.06 ± 2.42 **** | 48.09 ± 8.29 **** |
| LVMES | 103.5 ± 14.28 | 103.25 ± 15.66 | 103.92 ± 16.11 | 103.74 ± 14.28 | 101.73 ± 14.57 |
| LVMED | 97.33 ± 14.05 | 100.84 ± 16.52 | 100.30 ± 15.41 | 98.91 ± 15.83 | 97.69 ± 13.38 |

Abbreviations as in the Table 1 legend.

* $P < 0.05$;
**** $P < 0.0001$. 
3.4 | First-pass perfusion MRI

Mean heart rates during perfusion imaging were 337 ± 20 (Het) vs. 355 ± 17 (WT), \( P = 0.12 \). Examples of time series of myocardial first-pass perfusion images in both groups are provided in Figure 5A and the supporting information (supplemental material 3), illustrating the first passage of the contrast agent (CA) bolus after injection. The blood and myocardial signal are nearly nulled before CA injection by the saturation pulses. The arrival of the CA in the RV (~1.4 s/~1.4 s), the LV (~1.9 s/~2.0 s) and the myocardial influx (~4.1 s/~3.9 s) for Nexn WT/Het are clearly visible. The signal intensity-time curves of the LV lumen and LV myocardium in Nexn Het and WT mice are shown in Figure 5B,C. The first pass of contrast agent resulted in a prompt signal increase in the LV lumen. Four to five cardiac cycles later, a gradual signal increase in the LV myocardium could be appreciated, with a peak signal ~4 seconds after injection. Analysis of the upslopes in both groups yielded no significantly different values (Het, 0.051 ± 0.014; WT, 0.051 ± 0.011; \( P > 0.05 \)) for the LV myocardium (Figure 5D). The myocardial upslope for perfusion images reconstructed from data acquired over three heartbeats per image resulted in a trend (0.042 ± 0.009 [single] vs. 0.051 ± 0.012 [triple], \( P = 0.091 \), Figure 5E) but not yet significant reduction of the upslope steepness.

3.5 | Inter-study reproducibility of RT imaging

The inter-study reproducibility in general was excellent for all functional parameters (EDV, ESV, SV, EF, LVMED and LVMES) in RT1–RT2 protocols at rest, with ICC > 0.9 and CoVs < 10% (Table 3). Bland–Altman plots demonstrate a good inter-study reproducibility for RT imaging (Figure 6).
3.6 | Data quality analysis

No significant differences in SNR, CNR and image sharpness were observed for the investigated RT protocols RT1–RT2 (Figure 7). In comparison with the IG-HR, the SNR values were significantly reduced by ~ 40%, and a significant but only moderate reduction of the CNR of ~ 25% was observed for RT1–RT2. No differences in image sharpness were observed between RT1–RT2, but there was a clear reduction by ~ 35% compared with IG-LR, and more pronounced when compared with IG-HR.

4 | DISCUSSION

Even although an established imaging method, small animal cardiac MRI is still a time-consuming technique. Due to the high heart and respiratory rates, the resulting long acquisition time of gated or self-gated techniques limit its application in animal research. Imaging methods for accurate assessment of cardiac function are highly required by molecular biologists and geneticists. The aim of this study was to investigate RT functional cardiac imaging in the mouse model by applying tyGRASP to highly undersampled continuously acquired nongated radial data. The suggested approach could be applied successfully to the quantification of global cardiac function parameters in rest and pharmacological stress as well as to first-pass myocardial perfusion imaging.

After its introduction by Wundrak et al25 in 2016, tyGRASP has been applied in a few studies. Haris et al26 investigated fetal cardiac data of five volunteers at 1.5 T by using tyGRASP combined with self-gated cardiac cine MRI. Haji-Vayyadeh et al27 applied a balanced steady-state free
Results are shown as mean ± SD. RT protocol with RT1: LVMED/ES, end-diastolic/systolic left ventricular mass; SV, stroke volume. Abbreviations: CI, confidence interval; EDV/ESV, end-diastolic/systolic volume; EF, ejection fraction; ICC, intra-class correlation coefficient; precession sequence (b-SSFP) with tyGRASP in RT imaging. For preclinical research, Wech et al. investigated the application of radial generalized autocalibrating partially parallel acquisitions (GRAPPA) with large GA (111.25°) in RT imaging. In our study, we applied the tyGRASP to nongated RT functional imaging and first-pass perfusion imaging with single heartbeat temporal resolution in the Nexn-induced heart failure mouse model.

Mutations in Nexn are associated with cardiomyopathy, especially for the DCM, and result in dilated ventricular chambers and impaired contractility. Myocardial damage will finally lead to refractory heart failure and fatal arrhythmia. Compared with WT, a significantly increased EDV and ESV, and decreased EF, could be observed in the Het animals.

It could be proven that tyGRASP RT cardiac MRI in mice appears feasible for quantification of global functional parameters and enables a flexible number of projections for image reconstruction with a maximal temporal resolution of 16.8 ms (G^3 = 8), thereby preserving sufficient image quality for quantification of the global function parameters. Similar values without significant differences and excellent inter-study reproducibility were obtained by the RT protocols with a temporal resolution below 31.5 ms.

Cardiac stress MRI has been applied in preclinical research to detect cardiovascular abnormalities at an early stage. As RT imaging offers the rapid and continuous acquisition of image datasets, it might show advantages for the examination of the immediate response to physiological stress. The application of the tyGRASP technique in dobutamine stress examinations was tested in Nexn mice, enabling a complete stress examination within a few seconds. Under the effect of β-adrenergic stimulation, there was a significant decrease of both EDV and ESV, and also an obvious increase of EF. However, we could not detect apparent changes in SV.

With the acquisition protocol, 192 projections are required for fulfilling Nyquist's theorem. The RT1-RT4 schemes result in 24 (RT1), 12.8 (RT2), 8.4 (RT3) and 5.1 (RT4) -fold undersampling. Even although, because of the larger flip angle, the myocardium-blood contrast was enhanced and the object represented as somewhat sparse, the data are still strongly undersampled for all RT protocols. For compensation of the rising artifacts and improvement of SNR, we applied a CS reconstruction with TV filter along spatial and temporal domains. The parameters were chosen such that all images could be reconstructed without further adaptation of the regularization weighting, thus enabling the application of the technique to cohort studies. However, the presented approach still resulted in clearly reduced image quality when compared with the conventional self-gated techniques. However, the reduced image quality did not cause any significant differences in the functional parameters during rest as well as during pharmacological stress. Even although the surrounding structures are not well depicted in the images and remaining streak artifacts are not completely eliminated by the CS reconstruction, the relevant cardiac structures can be clearly followed over time, and beat-to-beat changes of functional parameters appear addressable.

Hoerr et al. reported beneficial properties of applying UTE to cardiac functional imaging. In particular, the reduction of flow- and susceptibility-induced artefacts was reported. In this work it was shown that the properties of tiny GA radial data acquisition could be used to provide sufficient image quality for global functional quantification. The combination with UTE for further artefact reduction is feasible in principle, but would halve the resulting temporal resolution and needs further investigation.

A general limitation of the proposed technique may arise from the only limited temporal resolution, which may limit its application for the identification of early diastolic dysfunction, which demands better temporal fidelity. Motaal et al. reported a CS approach in combination with

### Table 3

| Parameter          | S1       | S2       | Mean difference | Limits of agreement | ICC (95% CI) | Coefficient of Variations |
|--------------------|----------|----------|-----------------|----------------------|--------------|--------------------------|
| RT1 EDV            | 57.12 ± 7.26 | 58.04 ± 6.15 | −1.80          | −8.58 to 4.98        | 0.93 (0.48 to 0.99) | 3.17%         |
| RT1 ESV            | 13.24 ± 2.49 | 13.01 ± 2.22 | 1.35           | −7.83 to 10.53       | 0.95 (0.67 to 0.99) | 5.17%         |
| RT1 SV             | 43.88 ± 4.19 | 45.03 ± 4.63 | −2.71          | −9.33 to 3.90        | 0.92 (0.41 to 0.99) | 2.33%         |
| RT1 EF             | 76.93 ± 2.48 | 77.64 ± 2.61 | −0.91          | −2.29 to 0.46        | 0.96 (0.70 to 0.99) | 1.00%         |
| RT1 LVMED          | 101.38 ± 16.44 | 102.92 ± 14.29 | −1.81       | −8.10 to 4.48        | 0.97 (0.78 to 1.00) | 5.17%         |
| RT1 LVMES          | 103.52 ± 14.08 | 106.82 ± 14.95 | −3.04       | −11.32 to 5.23       | 0.92 (0.44 to 0.99) | 5.50%         |
| RT2 EDV            | 56.47 ± 6.10 | 56.59 ± 4.01 | −0.17          | −6.28 to 5.94        | 0.91 (0.36 to 0.99) | 3.83%         |
| RT2 ESV            | 13.38 ± 2.48 | 13.31 ± 1.90 | 1.32           | −11.22 to 13.86      | 0.91 (0.35 to 0.99) | 4.50%         |
| RT2 SV             | 43.09 ± 3.85 | 43.29 ± 2.93 | −0.59          | −5.40 to 4.22        | 0.91 (0.39 to 0.99) | 3.67%         |
| RT2 EF             | 76.46 ± 2.33 | 76.54 ± 2.49 | −0.42          | −2.67 to 1.84        | 0.93 (0.46 to 0.99) | 0.67%         |
| RT2 LVMED          | 99.31 ± 16.96 | 101.90 ± 13.63 | −3.07       | −12.73 to 6.58       | 0.93 (0.52 to 0.99) | 3.17%         |
| RT2 LVMES          | 103.35 ± 16.72 | 105.70 ± 14.27 | −2.64       | −10.82 to 5.55       | 0.93 (0.47 to 0.99) | 4.83%         |

Abbreviations: CI, confidence interval; EDV/ESV, end-diastolic/systolic volume; EF, ejection fraction; ICC, intra-class correlation coefficient; LVMED/ES, end-diastolic/systolic left ventricular mass; SV, stroke volume.

Results are shown as mean ± SD. RT protocol with RT1: G^3 = 8, Δt = 16.8 ms; RT2: G^3 = 15, Δt = 31.5 ms.
self-gating for achieving up to 90 cardiac frames. Due to the undersampling properties of the suggested tyGA approach, its combination with the reported reconstruction technique may allow for further reduction of the acquisition time.

Myocardial perfusion MRI is a valuable and safe examination to evaluate the viability of myocardium in patients with suspected or diagnosed ischemic heart disease.\textsuperscript{39-41} In preclinical research, with a high heart rate and fast circulation time in particular, the mouse model requires perfusion imaging protocols with high temporal resolution and adequate spatial resolution for the assessment of regional myocardial influx. Coolen et al.\textsuperscript{42} and van Nierop et al.\textsuperscript{43} estimated the quantitative regional myocardial perfusion values with an ECG-triggered, segmented saturation-recovery FISP sequence. They reported a temporal resolution of three cardiac cycles (300–400 ms). Naresh et al.\textsuperscript{44} reported a dual-contrast, saturation-recovery, first-pass perfusion sequence with motion-compensated CS reconstruction yielding single heartbeat temporal resolution. We could show single heartbeat temporal resolution by applying a CS reconstruction with TV filter as sparsity constraint. Even although it was not statistically significant, the myocardial upslope of one image per three cardiac cycles was relatively lower than one image per cardiac cycle, indicating a potential benefit for inflow and washout visualization. The normalized upslopes clearly reveal the characteristic difference between blood and

\textbf{FIGURE 6} Bland–Altman plots with limits of agreement (1.96 standard deviation) demonstrate the inter-study reproducibility of RT protocols in nexilin wild-type mice: (A) RT protocol with $G_3^7 = 8$, $\Delta t = 16.8$ ms, and (B) $G_4^7 = 15$, $\Delta t = 31.5$ ms
myocardium. As the Nexn-KO induced DCM is a nonischemic heart disease, we did not find a decreased perfusion region of myocardium in first-pass perfusion imaging at rest.

In comparison with the established methods, the main advantage of the proposed highly accelerated approach results from its capabilities of ungated RT CMR imaging. For quantification of the cardiac function, a substantial decrease of scan time down to a few seconds for full left ventricular coverage can be realized, thus relaxing the load of circulatory challenged animals and ensuring stable conditions during monitoring, eg drug-induced changes. In the presence of irregular animal motion, the RT approach may still provide data sufficient for quantification of the cardiac function. Compared with other RT approaches, in tyGRASP, the combination with tiny GAs enables a highly flexible choice of the number of spokes used for the reconstruction of a single time frame thus enabling trading of spatial vs. temporal resolution, which might be of particular interest in perfusion imaging.

There are some limitations to this study. Due to the nature of the CS reconstruction, the interpretation of the resulting SNR, CNR and image sharpness differences should be made with care, always considering a severe impact of the iterative regularized nature of the CS. However, the nonsignificant differences for the functional parameters clearly indicate the potential of the RT approach. During the perfusion acquisition, we neglected respiratory motion-induced displacement since we did not notice any substantial impact. The tiny fluctuation of the heart rate may result in a slight change of the imaged cardiac phase, and some remaining beat-to-beat differences of the cardiac phase can be appreciated in the supplemental video (see the supporting information). No quantification of the perfusion has been performed and only qualitative assessment was proven. Further, the approach was only tested on a Nexn-induced heart failure mouse model and the sensitivity to evaluate tiny changes in perfusion in mouse models with myocardial infarction needs further work.

For future perspectives, our approach contains the potential to investigate myocardial perfusion with multiple slices. By acquiring a fixed number of projections from different slices in each cardiac cycle, multiple slices perfusion imaging may be achieved in a single scan.

5 | CONCLUSIONS

RT cardiac tyGRASP in mice appears feasible with sufficient image quality for quantification of functional parameters. The tyGRASP approach enables a flexible number of projections for image reconstruction thus offering the possibility for cardiac rate- and phase-dependent adjustment of the temporal resolution, which might further optimize the resulting image quality. This protocol can be applied to pharmacological stress or perfusion imaging. It has the potential to reveal RT and inter-beat variations of the cardiac contraction. In combination with cardiac triggering and saturation recovery contrast, single heartbeat first-pass perfusion data appear feasible with high temporal resolution.
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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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