Published in final edited form as:

J Am Soc Echocardiogr. 2010 August ; 23(8): 887–897. doi:10.1016/j.echo.2010.05.017.

An Evaluation of Transmitral and Pulmonary Venous Doppler Indices for Assessing Murine Left Ventricular Diastolic Function

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Abstract

With the continued development of genetically engineered mouse models of cardiac disease, further advancement of non-invasive techniques for evaluating cardiac diastolic dysfunction in these models would be valuable. Therefore, we performed comprehensive transmitral and pulmonary venous Doppler echocardiographic studies to devise novel indices of diastolic function in a mouse model with cardiac hypertrophy, which were validated against invasively measured hemodynamic parameters. We examined 10 *HopX^{Tg}* transgenic mice with diastolic dysfunction and 10 age-matched controls sedated with 1–2% isoflurane (male, age 14–18 weeks). These studies revealed the acceleration time of the transmitral Doppler E-wave was the best Doppler parameter for unmasking LV diastolic dysfunction in *HopX^{Tg}* mice. This is the first study to assess the utility of the acceleration time of the E wave and pulmonary venous Doppler echocardiography as a primary diagnostic modality for assessing murine diastolic function.

Keywords

Diastolic function; transmitral Doppler echocardiography; pulmonary vein Doppler echocardiography; genetically engineered mouse model; invasive hemodynamics

Over the past two decades, the prevalence of heart failure due to diastolic dysfunction has been gradually rising. Despite the growing incidence of this disorder, no effective therapies exist to treat the disease, halt its progression or reduce the associated mortality¹. The development of transgenic and gene targeting techniques in mice has significantly expanded our understanding of disease causing pathways, and increased the availability of faithful models for evaluating clinical therapies. Several groups have developed genetically

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DISCLOSURES

The authors have no competing financial interests to disclose.

engineered mice with impaired myocardial relaxation and some investigators have used these models to assess therapeutic strategies for treating myocardial diastolic dysfunction^{2–4}. Transmitral Doppler echocardiography has been routinely used to identify left ventricular diastolic dysfunction in patients^{5, 6}, and many of these indices have been validated against invasive hemodynamic measurements in small animal models^{7, 8}. In addition, several groups have used high-resolution ultrasound systems to study mouse cardiac structure and function^{9–12}. This technology may provide better spatial and temporal resolution compared with conventional echocardiographic equipment. However, problems related to the complexity of interpreting the transmitral flow profile still exist, and some of the better established clinical indices may need to be re-evaluated for their relevance in murine models due to interspecies differences in cardiac physiology. The main difference that affects interpretation of the murine transmitral inflow profile is the higher heart rate which often fuses the E-wave and A-wave. Although pulmonary venous Doppler echocardiography is routinely used in the clinical setting to assess diastolic function, no detailed studies have reported the utility of pulmonary venous Doppler echocardiography for evaluating murine diastolic function. To address these questions we performed a comparative study of transmitral and pulmonary venous Doppler echocardiography, and compared these with invasive measurements of LVEDP, $-dP/dt_{\min}$ and tau in a genetically engineered mouse model with diastolic dysfunction.

These investigations revealed that the acceleration time of the transmitral Doppler E-wave is the best parameter for assessing LV diastolic dysfunction in *HopX^{Tg}* mice. This is the first study to demonstrate that the acceleration time of the transmitral Doppler E-wave may be useful for assessing diastolic function in murine models. While this study did not uncover any pulmonary venous Doppler parameters that correlated with invasive parameters of diastolic dysfunction in *HopX^{Tg}* mice, the comprehensive evaluation of pulmonary venous Doppler analysis described in this report will likely be useful for assessing diastolic dysfunction in other mouse models.

MATERIALS AND METHODS

Animals

Homeodomain-only protein transgenic mice (*HopX^{Tg}*) develop cardiac hypertrophy with cardiomyocyte enlargement due to HDAC recruitment and have been previously described¹³. We studied 10 *HopX^{Tg}* and 10 age-matched wild-type (WT) mice weighing 25 to 35 grams that were 14–18 weeks-old. All protocols used conformed to the guidelines established by the Association for the Assessment and Accreditation of Laboratory Animal Care and were approved by the University of Pennsylvania Animal Care and Use Committees. This investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Transthoracic echocardiographic imaging

A comprehensive transthoracic 2-dimensional (2-D) and Doppler echocardiographic study was conducted using a high-resolution (30-MHz) Vevo 770 imaging system (VisualSonics Inc., Toronto, Canada) as previously described^{13, 14}. Mice were lightly anesthetized with a mixture of 1–2% isoflurane gas and 100% oxygen while supine on a heated platform. Several studies have compared different anesthetic regimens upon murine cardiovascular function, and inhaled anesthetics appear to be the most advantageous since they have the least cardiodepressive effects with a relatively short half-life^{15–17}. Furthermore, we compared 1% isoflurane versus 2% isoflurane upon the echocardiographic and invasive parameters we measured in a subset of *HopX^{Tg}* and wild-type mice, and found no significant

differences in these measurements at either dose, suggesting the cardiovascular effects of isoflurane are close to maximal at a concentration of 1% in these mice (Supplementary Table 1). The mouse's core temperature was monitored using a rectal temperature probe, and an infrared heating lamp was used to maintain body temperature at $37\pm0.5^{\circ}\text{C}$ throughout the procedure. An ECG signal was also monitored through electrode pads on the heated platform. Chest hairs were removed using a chemical depilator (Nair) to minimize ultrasound attenuation. The ultrasound probe (RMV-707B) was placed on the mouse's chest using warm ultrasound gel as a coupling medium.

Two-dimensional echocardiography

Two-dimensional images were recorded in the parasternal long- and short-axis views to guide M-mode recordings obtained at the mid-ventricular level. Wall thicknesses of the interventricular septum (IVS) and left ventricular (LV) posterior wall (LVPW), as well as the LV diastolic and systolic internal dimensions (LVIDd and LVIDs, respectively) were measured, and the average values from both views are reported. LV systolic function was computed from the M-mode measurements according to the recommendations of the American Society of Echocardiography Committee¹⁸. Relative wall thickness (RWT) was calculated using the formula: $(\text{IVSd} + \text{LVPWd}) / \text{LVIDd}$, and left atrial (LA) area was measured in the four-chamber view during LV systole using the polygon ROI (region of interest) area measurement tool in the Visualsonics software suite (Visualsonics Standard Measurements, version 23) with the program's B-mode generic measurement feature.

Transmitral Doppler echocardiography

Pulsed Doppler studies of LV diastolic function were performed in the apical 4-chamber view with the Doppler cursor oriented parallel to the long-axis plane of the left ventricle. The sample volume was placed just below the level of the mitral annulus and adjusted to render the highest early diastolic flow velocity peak of the transmitral Doppler flow signal. There was often a need for angle correction which was always less than 20 degree. The early and late diastolic peak velocity (E, A) and their ratio (E/A) were derived from the transmitral Doppler waveform. Left ventricular systolic intervals of the isovolumic contraction time (IVCT), the ventricular ejection time (ET), diastolic intervals of the isovolumic relaxation time (IVRT) and the acceleration and deceleration times of the E-wave (E_{AT} and E_{DT} , respectively) were also derived from the transmitral Doppler waveform¹⁹.

Pulmonary venous Doppler echocardiography

The pulmonary vein (PV) was visualized by orientating the transducer in the parasternal long-axis view with the left atrium, left ventricle and aorta in the field (Figure 1A–B). The transducer was then moved laterally, rotated clockwise by 10–20 degrees and the beam was then oriented slightly inferiorly and anteriorly to visualize the pulmonary vein (Figure 1C–D). The single PV connects to the LA close to the LV atrioventricular groove (Figure 1D). The PV Doppler waveform was obtained by placing the Doppler sample volume ~1 mm away from the LA-PV junction with an angle correction of less than 15 degrees^{13, 14}. To locate the pulmonary vein we would first identify the aorta in the parasternal long-axis view, and then tilt the transducer posteriorly until the aorta disappeared and the pulmonary vein came into the field. In mice the coronary sinus is in close anatomic proximity to the PV and so the coronary sinus could be mistaken for the PV while trying to obtain Doppler recordings. This type of confusion may be avoided by analyzing the Doppler spectra, where blood flow in the coronary sinus is affected to a much larger degree by respirations than in the PV. Pulmonary venous flow velocity and the velocity-time integral (VTI) of all waves were then measured. All Doppler parameters are reported as the average value obtained from three consecutive cardiac cycles.

Invasive hemodynamics

Protocols for invasive hemodynamic recordings have been described before^{13, 20}. Briefly, anesthesia was achieved using 1–2% isoflurane with 100% oxygen by ventilation and core body temperature was maintained at 36–37°C using a heating pad and rectal temperature probe. Intracardiac pressures were recorded with a 1.4 Fr micro-tip catheter (SPR-839; Millar Instruments, Houston, TX), and zeroed in a saline bath prior to obtaining measurements from each animal. The catheter was inserted via the right carotid artery into the left ventricle to record intracardiac pressures. Signals were digitized at 2-kHz using a PowerLab/16 SP A/D converter (ADInstruments Ltd., Mountain View, CA) and stored on the hard drive of a Core Duo-based PC computer for off-line analysis. Data were analyzed using the PVAN3.2 analysis suite (Millar Instruments) and normalized to the mean arterial pressure to account for load-dependency. The LV relaxation time constant (τ) was calculated by two different methods: 1) the Weiss method (τ (w)): regression of log (pressure) versus time and, 2) the Glantz method (τ (g)): regression of dp/dt versus pressure.

Intra- and inter-observer variability

To evaluate intra-observer and inter-observer variability of Doppler echocardiographic measurements, one same observer and two independent observers re-measured 12 sets of values for E_{AT} , pulmonary venous first diastolic wave velocity and IVRT in 12 animals using the same cardiac cycles.

Statistical analysis

All values are reported as the mean $\pm$ 1 standard deviation. Differences between groups were analyzed using 2-tailed Student's t-test. Linear regression analysis was used to determine correlations between echocardiographic and invasive relaxation parameters. Agreement between two measurements was determined according to the method of Bland and Altman²¹. A p-value < 0.05 was considered significant for all analysis (Prism 5, GraphPad Inc, San Diego, CA). Data were not corrected for multiple comparisons to reduce Type I error due to the risk of introducing Type II error²².

RESULTS

We previously studied *HopX^{Tg}* mice and found most of these animals develop LV hypertrophy¹³ with diastolic dysfunction by the age of 14–18 weeks. Therefore, we used these mice as a model of LV hypertrophy and diastolic dysfunction for these studies. We confirmed all *HopX^{Tg}* mice used in this investigation had LV hypertrophy, as denoted by a significant increase in diastolic relative wall thickness and LV mass-to-body weight ratio (Table 1).

Evaluation of LV relaxation in *HopX^{Tg}* mice using invasive hemodynamics

While *HopX^{Tg}* mice clearly develop LV hypertrophy, we next sought to determine the extent to which LV diastolic function was affected in these animals. Invasive hemodynamic recordings revealed *HopX^{Tg}* mice had reduced LV relaxation compared to wild-type control mice, where τ (g) and LVEDP were significantly higher and $-dp/dt_{min}$ was much lower (Fig. 2A–B & Table 2). These data confirm that *HopX^{Tg}* mice with LV hypertrophy have impairments in LV relaxation. Furthermore, measurements of the LV peak pressure and $+dp/dt_{max}$ revealed that LV systolic function is preserved in *HopX^{Tg}* mice (Table 2).

Evaluation of LV relaxation in *HopX^{Tg}* mice by Doppler echocardiography

Transmitral Doppler echocardiography provided evidence for reduced LV relaxation in *HopX^{Tg}* mice as revealed by a decrease in the E-wave flow velocity and E/A ratio, and an increase in the acceleration time of the transmitral early diastolic peak flow velocity (E_{AT}) (Fig. 2C–D & Table 2). On the other hand, the deceleration time of the transmitral early diastolic peak flow velocity (E_{DT}), which is a function of LV compliance and typically affected in later stages of diastole dysfunction, was not different between WT and *HopX^{Tg}* mice (Table 2).

Doppler interrogation of the pulmonary vein revealed the waveform was composed of a small S-wave, two forward D-waves (D1 and D2) and a small, reversed *a*-wave in all WT mice examined. While we could obtain pulmonary venous flows from every mouse we studied, approximately 50% of the *HopX^{Tg}* mice examined did not have a D2 wave. Lack of a D2 wave in half of the *HopX^{Tg}* mice probably accounts for the slight, yet significant, decrease in the pulmonary VTI during diastole (PV VTId) in *HopX^{Tg}* mice compared to control littermates (Fig. 2E–F, Table 2).

Comparison of LV relaxation by echocardiography versus invasive hemodynamics in *HopX^{Tg}* mice

In order to validate the Doppler echocardiographic indices for assessing diastolic function in mice, we compared several non-invasive measurements of LV filling and relaxation with invasively measured parameters of LV relaxation. In this model, we found that the E_{AT} by Doppler echocardiography, and RWT and LA area by non-Doppler echocardiography correlated well with $-dp/dt_{min}$, tau (g) and LVEDP (Fig. 3 & Table 3). In contrast, the IVRT did not correlate well with $-dp/dt_{min}$ and the E/A ratio did not show good correlation well with any of the invasive parameters (Table 3).

Intra- and inter-observer variability

We saw good agreement between measurements taken by the same observer for Doppler echocardiographic values. It appears there was no difference (0.0 ms) in repeated measurements of IVRT taken by the same observer (Fig. 4A–B). Similarly, when measurements were taken by two independent observers, the mean difference between values was -0.85 ms for IVRT (Fig. 4C–D). We saw similar agreement between values obtained for the acceleration time of the Doppler E-wave for the same (Fig. 4E–F) and two independent observers (Fig. 4G–H), as well as those measured for the pulmonary vein D1-wave flow velocity (Fig. 5).

DISCUSSION

To the best of our knowledge, this is the first study to investigate the utility of the acceleration time of the transmitral Doppler E-wave and the Doppler pulmonary venous flow for assessing murine LV diastolic function.

Transmitral Doppler echocardiography for assessing LV diastolic function in mice

The transmitral Doppler indices: E-wave flow velocity, E/A ratio, IVRT and E_{DT} are the most commonly used values for assessing diastolic function in the clinical setting, and we show in this study that besides E-wave flow velocity and E/A ratio, E_{AT} may also be useful for assessing diastolic function in mice. E_{AT} has previously been reported as a sensitive, transmitral Doppler parameter that is useful for assessing left ventricular diastolic function in patients with diabetes or coronary artery diseases^{23, 24}. To our knowledge, this is the first study to demonstrate that the acceleration time of the transmitral Doppler E-wave may be useful for assessing diastolic function in murine models.

In human hearts, the E/A ratio provides general information about the LV filling characteristics of normal and diseased hearts, where an E/A ratio greater than 1.2 has been accepted as a reference for staging diastolic dysfunction. Clinically, there are three recognized abnormal LV filling patterns: *impaired relaxation*, *pseudonormalization* and *restriction*²⁵. The least abnormal pattern is *impaired relaxation*, and is diagnosed by a reduction in the E/A ratio due to lower early diastolic filling with increased late diastolic filling due to the relatively larger contribution from atrial contraction. With disease progression, LV compliance is further reduced and left atrial pressure increases, which appears to hemodynamically counteract impaired LV relaxation. Increased early transmitral pressure gradient results in an LV filling pattern that appears normal, but is in fact *pseudonormalized*. Finally, in patients with advanced disease and severe reduction in LV compliance, high intracardiac pressures cause LV filling to show a *restrictive* pattern, characterized by a E/A ratio larger than 2. Note that these three abnormal LV filling patterns are defined subjectively, whereas impaired LV filling is a continuous process that lacks clearly delineated boundaries.

While values of the E/A ratio that correspond to various stages of clinical diastolic function are based upon evaluations from hundreds of normal and diseased patients, the usefulness and range of values that correspond to murine diastolic function have not been established. In the present study, if we take the range of the mean and standard deviation of the E/A ratio in WT mice as the reference, where the E/A ratio is greater than 1.22 (mean-2SD) and less than 1.64 (mean+2SD), the transmitral Doppler flow patterns in 8 out of 10 *HopX^{Tg}* mice would be assigned to impaired LV relaxation, where the E/A ratio of these 8 mice were all less than or equal to 1.20. In addition, for 8 these transgenic mice the intraventricular septum and LV ventricular free walls were significantly hypertrophied, the E_{AT} was prolonged, tau (g) and LVEDP were significantly higher and $(-dp/dt_{min})/LVSP$ was lower, which further confirms the finding of impaired LV relaxation. However, findings from the two other *HopX^{Tg}* mice suggest the E/A ratio may not be the best parameter for detecting diastolic dysfunction in some murine models, whereas prolongation of the E_{AT} may be more sensitive in this regard. For example, consider that the other two *HopX^{Tg}* mice had an E/A ratio of 1.44 and 1.75, respectively, which suggests these animals have impaired relaxation since these ratios are greater than 1.20 but less than 2 (which is the upper limit defined in clinical studies). However, it is very likely these two mice actually have pseudonormalization since they both displayed significant LV hypertrophy, a low $(-dp/dt_{min})/LVSP$ and an elevated LVEDP. Importantly, the E_{AT} was significantly longer in these two mice which is also consistent with pseudonormalization. The acceleration time (AT) may be more sensitive for detecting early diastolic dysfunction in mice because this parameter is acquired during the initial phase of left ventricular filling to the peak, which appears to be the most severely affected phase in this model. It is also possible that the IVRT may be within the normal range with a pseudonormalized filling pattern, as we saw with these two *HopX^{Tg}* mice. In addition, we found a prolonged E_{AT} correlates well with invasive hemodynamic parameters of diastolic dysfunction, including tau (g), $dp/dt_{min}/LVESF$ and LVEDP. Therefore, prolongation of the E_{AT} may not only be sensitive for detecting early stages of diastolic function, but appears to add incremental value over that provided by the E/A ratio and IVRT for detecting later stages of diastolic dysfunction in murine models.

Since E_{DT} is a function of LV compliance and usually appears abnormal during late stages of diastolic dysfunction, the fact that we found no difference in E_{DT} between WT and *HopX^{Tg}* mice suggests diastolic impairment in most *HopX^{Tg}* mice (age 14–18 weeks) probably represents an early stage of impaired relaxation. Whether older *HopX^{Tg}* mice or other transgenic mouse models with advanced diastolic dysfunction would demonstrate other transmitral Doppler flow patterns such as pseudonormalization or restriction, similar to those observed in the clinical setting, deserves further investigation.

It is also important to note that the transmitral flow velocity at the start of atrial systole, known as the “E at A velocity”, will often show partial fusion of the early and late diastolic filling waves at the faster heart rates typical of murine models²⁵. Partial fusion of the diastolic filling waves may artificially augment A-wave velocity, but fusion of the filling waves probably does not contribute significantly to the close-to-1 E/A ratios we observed in *HopX^{Tg}* mice since the heart rates between these groups were not different (Table 2). Interestingly, a monophasic Doppler filling pattern often reflects compromised ventricular diastolic function in human fetal hearts²⁶, and fusion of the E- and A-waves may also be an indicator of diastolic dysfunction in genetically engineered murine models at comparable heart rates with control littermate mice. In this regard, we have observed a single low peak transmitral Doppler flow wave in other mouse models that have both impaired diastolic and systolic function (i.e. LPS-induced myocarditis and LAD-ligation induced ischemic cardiomyopathy).

Pulmonary venous Doppler flow indices for assessing LV diastolic function in mice

In humans, pulmonary venous flow is often combined with transmitral Doppler flow indices to assess diastolic function. The use of pulmonary venous Doppler parameters to assist with diagnosing diastolic function in mice has not been evaluated or widely applied, perhaps due to the differences that exist in the pulmonary vein anatomy and physiology between humans and mice. The fact mice usually have a single pulmonary vein²⁷ and humans have four, requires increased technical skill to locate the single murine pulmonary vein which is not often visible in the normal four-chamber apical view. In addition, the pulmonary venous Doppler flow waveforms differ significantly between the two species. In humans, pulmonary venous Doppler flow waveforms are usually composed of a forward S1-wave (during early LV systole), an S2-wave (during late LV systole) and a D-wave (during early LV filling) with a small reversed *a*-wave (during atrial contraction). However, in mice the pulmonary venous waveform usually consists of a small single S-wave, two forward D-waves (D1 and D2) and a tiny reversed *a*-wave (Fig. 2E–F). The VTI of the pulmonary venous flow patterns suggests that about twice the volume of forward flow occurs during ventricular diastole compared with systole in mice, so it makes sense the D-wave may be more sensitive for assessing diastolic function in mice. Furthermore, in normal humans the A-wave duration of the transmitral Doppler waveform (MV *A*dur) is usually similar to that of the reversed *a*-wave of the pulmonary venous Doppler waveform (PV *a*dur)²⁸. However, with regards to interpreting the pulmonary vein flow profile, humans typically have four individual pulmonary veins that are each much smaller in diameter than the left atrium; so the pressure difference between each pulmonary vein is relatively large and produces a robust flow profile. On the other hand, the single murine pulmonary vein is relatively large in relationship to its left atrium, so the pressure differential is lower between these structures in mice. This difference results in a less robust flow profile that may equilibrate more quickly and act to shorten the duration of the murine PV *a*-wave, while duration of the murine MV *A*-wave is less effected by murine anatomy (Figure 2B–D)²⁹.

While we did not detect significant differences in the pulmonary vein flow profiles that were diagnostic of diastolic dysfunction in *HopX^{Tg}* mice, the description and practical application of this technique is likely to be useful for assessing myocardial relaxation in other murine models. We used a high-frequency array for these studies which obviously has better resolution for detecting fine structures in the murine heart compared to clinically available arrays centered on 13–15 MHz. However, the murine pulmonary vein is a relatively large structure (~1 mm in diameter) that may be detectable with more commonly available 13–15 MHz arrays. Still, given that the resolution of these lower frequency systems range from 100 μ m to 450 μ m^{30, 31}, it remains to be shown just how well the mouse pulmonary vein can be imaged with 13–15 MHz arrays.

LIMITATIONS

We did not explore the use of several state-of-the-art techniques, including Doppler tissue imaging and 3-D spectral tracking imaging in this study, due to lack of the necessary software on our system, but these modalities may provide valuable information for assessing cardiac function in murine models. However, validation of several novel Doppler indices of murine diastolic function, using more commonly available equipment and modalities should allow most laboratories to immediately exploit the value of these findings. Another limitation of the present study is we studied a single model with diastolic dysfunction. Further validation of these findings in different murine models with diastolic function is needed, and may show pulmonary venous wave differences are significant in some models with varying degrees of diastolic dysfunction.

SUMMARY

We have found several Doppler echocardiographic indices of diastolic function correlate well with invasive hemodynamic measurements in *HopX^{Tg}* mice. The main finding of this study is that the acceleration time of the transmitral Doppler E-wave is a reliable parameter for assessing LV diastolic function in murine models. In addition, the combination of all the parameters we assessed by non-invasive echocardiography, and then validated with invasive hemodynamic parameters, show that *HopX^{Tg}* mice manifest an early stage of diastolic dysfunction at 14–18 weeks of age, which may worsen with aging.

Acknowledgments

We thank Jonathan Epstein for kindly providing us with *HopX^{Tg}* mice.

GRANTS

This work was supported by grants from the NIH, the McCabe Foundation and the Barra Foundation to V.V.P.

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Fig. 1. Schematic diagram exemplifying the technique used to locate and visualize the murine pulmonary vein. (A) The transducer was first positioned to obtain a standard parasternal long axis view. (B) Representative image displaying the left atrium (LA), left ventricle (LV) and aorta (Ao) in the field with the transducer positioned as shown in panel A. (C) To image the pulmonary vein the transducer was moved a little laterally, rotated clockwise 10 to 20 degrees and the beam was then slightly oriented anteriorly and inferiorly. (D) Representative image showing the pulmonary vein (PV) that would appear with the LV and LA, just after the Ao would disappear from the field, with the transducer oriented as in panel C.

Fig. 2.

Left ventricular relaxation indices in *HopX^{Tg}* and wild-type mice. Invasive hemodynamic recordings reveal *HopX^{Tg}* mice have reduced LV relaxation compared to wild-type mice where $-dP/dt_{\min}$ is significantly decreased (A, B) in *HopX^{Tg}* mice. Similarly, Doppler echocardiography provides evidence for reduced LV relaxation in *HopX^{Tg}* mice, which shows prolongation in the E-wave acceleration time (D, the interval between the two vertical solid lines) compared to wild-type littermates (C). Note that the E/A ratio appears normal in this *HopX^{Tg}* mouse suggesting it is pseudonormalized. Furthermore, the A-wave duration in the transmitral Doppler profile (the interval between the two dotted lines) and the *a*-wave duration in the pulmonary venous flow profile (the interval between the two dashed lines) are distinctly different in both wild-type and *HopX^{Tg}* mice (C-F). E: transmitral early

diastolic peak flow velocity; A: transmitral late diastolic peak flow velocity; S: pulmonary venous flow during LV systole; D: pulmonary venous flow during LV diastole; a: pulmonary venous flow during left atrial contraction.

Fig. 3.

Linear regression analysis of LV relaxation indices assessed by echocardiography versus invasive hemodynamics in *HopX^{Tg}* and wild type mice. Both $-dp/dt_{min}$ and τ g appear to correlate strongly with E_{AT} (A, B), but less well with the E/A ratio (C, D), IVRT (E, F) and PV VTID (G, H). E_{AT} : transmitral Doppler E-wave velocity acceleration time; E/A ratio: transmitral early-to-late diastolic peak flow velocity ratio; IVRT: isovolumic relaxation time; PV VTID: pulmonary venous velocity-time integral during diastole. Note there are overlapped solid dots in panels A, B, D, F and G that makes it appear as if only nineteen data points are shown: blue circles = wild-type mice; red circles = *HopX^{Tg}* mice.

Fig. 4.

Intra- and inter-observer variability of Doppler interval measurements. Linear regression analysis shows good agreement between measurements for parameters taken by the same observer for IVRT (A, B) and E_{AT} (E, F). Similarly we saw good agreement exists for measurements taken by two independent observers for IVRT (C, D) and E_{AT} (G, H).

Fig. 5. Intra- and inter-observer variability of pulmonary venous velocity measurements. Linear regression analysis shows good agreement between measurements by the same observer for PV D1 velocity (A, B). Similarly good agreement also exists for measurements taken by two independent observers for PV D1 velocity (C, D).

Table 1Morphometric measurements of wild-type and *HopX^{Tg}* mice

	Relative wall thickness	LV mass index (mg/g)	Heart weight (mg)	Heart weight/tibia length (mg/mm)
Wild-type	0.32±0.03	2.62±0.18	125.12±14.50	7.23±0.68
<i>HopX^{Tg}</i>	0.71±0.12	3.92±0.64	256.61±33.83	14.35±1.99
p-value	< 0.0001	<0.0001	<0.0001	<0.0001

Values are presented as the mean ± SD.

Table 2Echocardiographic and hemodynamic indices from wild-type and *HopX^{Tg}* mice

	Wild-type (n=10)	<i>HopX^{Tg}</i> (n=10)	p-value
2-D Echocardiographic indices			
IVST (mm)	0.64±0.06	1.25±0.16	<0.0001
LVPWT (mm)	0.62±0.04	1.19±0.13	<0.0001
LVIDd (mm)	3.90±0.28	3.45±0.28	0.003
LVIDs (mm)	2.71±0.38	1.64±0.41	<0.0001
LVEF (%)	60.5±6.1	83.8±7.1	<0.0001
LVFS (%)	32.0±4.2	53.8±8.2	<0.0001
LVEDV (μl)	70.4±6.8	52.8±10.5	<0.0001
LVESV (μl)	29.8±6.9	8.9±5.6	<0.0001
SV (μl)	40.6±4.7	43.8±7.1	0.242
CO (ml/min)	19.7±3.6	20.2±2.8	0.730
LA (mm ²)	3.7±0.9	6.3±0.9	<0.0001
Transmitral Doppler indices			
E flow velocity (cm/s)	81.2±7.2	54.75±10.62	<0.0001
A flow velocity (cm/s)	57.2±7.1	42.7±8.8	0.006
E/A ratio	1.4±0.1	1.19±0.2	0.012
IVRT (ms)	12.3±2.5	15.6±5.6	0.082
E _{AT} (ms)	11.9±0.7	17.7±1.1	<0.0001
E _{DT} (ms)	27.5±9.6	31.0±7.8	0.072
IVCT (ms)	10.7±3.4	9.7±2.8	0.229
ET (ms)	41.6±4.4	45.5±8.2	0.456
Vel LVOT (cm/s)	28.7±9.5	152.3±46.8	<0.0001
Heart rate (bpm)	484±68	465±44	0.445
Pulmonary venous Doppler indices			
PV a-wave velocity (cm/s)	-4.7±1.6	-6.0±3.6	0.306
PV S-wave velocity (cm/s)	22.8±5.4	20.0±3.9	0.197
PV D1-wave velocity (cm/s)	48.5±6.5	43.9±12.6	0.279
PV D2-wave velocity (cm/s)	34.8±9.0	28.0±9.5	0.202
PV S/D1	0.47±0.10	0.48±0.12	0.934
PV S/D2	0.69±0.24	0.78±0.32	0.405
PV VT1a (cm)	0.03±0.01	0.04±0.01	0.127
PV VTIS (cm)	0.62±0.16	0.57±0.19	0.523
PV VTID (cm)	1.90±0.37	1.56±0.24	0.028
Hemodynamic indices			
-dP/dt _{min} (mmHg/sec)	-7950±1490	-6240±1140	0.010
(-dP/dt _{min})/LVSP (s ⁻¹)	-74.4±10.9	-57.0±14.9	0.007
Tau g (ms)	10.0±1.0	15.8±4.0	<0.001
Tau w (ms)	7.5±1.1	9.3±2.9	0.099
LVEDP (mmHg)	8.1±4.0	13.6±4.6	0.011

	Wild-type (n=10)	<i>HopX^{Tg}</i> (n=10)	p-value
Peak LV pressure (mmHg)	112.3±9.5	126.5±9.3	0.335
+dP/dt _{max} (mmHg/sec)	11080±2800	10890±1880	0.861

Values are presented as the mean ± SD. IVST: Interventricular septum thickness; LVPWT: Left ventricular posterior wall thickness; RWT: Relative wall thickness; LVIDd and LVIDs: Left ventricular internal diameter during diastole and systole, respectively; LVEF: Left ventricular ejection fraction; LVFS: Left ventricular fractional shortening; LVEDV and LVESV: Left ventricular end-diastolic and end-systolic volume, respectively; SV: Stroke volume; CO: Cardiac output; E/A ratio: Transmitral early-to-late diastolic peak velocity ratio; MV VTI: Velocity-time integral of transmitral Doppler flow; IVRT: Isovolumic relaxation time; EAT: Acceleration time of transmitral early diastolic peak flow velocity; EDT: Deceleration time of transmitral early diastolic peak flow velocity; IVCT: Isovolumic contraction time; ET: Ejection time; Vel LVOT: Left ventricular outflow tract velocity; PV VTla, PV VTIS and PV VTID: Velocity-time integral of pulmonary venous flow during left atrial systole; LV systole and LV diastole, respectively; LVSP: Left ventricular systolic pressure.

Table 3

Correlation of echocardiographic and hemodynamic indices

Index	LVEDP (mmHg)		(-dP/dt _{min})/LVSP (s-l)		Tau (g) (ms)	
	r	p-value	r	p-value	r	p-value
E/A ratio	-0.05	0.822	0.05	0.819	-0.28	0.239
E _{AT} (ms)	0.60	0.005	0.60	0.005	0.74	<0.001
E _{AT} /R-R interval	0.56	0.010	0.55	0.012	0.78	<0.0001
E flow velocity (cm/s)	-0.25	0.295	-0.23	0.335	-0.50	0.026
IVRT (ms)	0.37	0.106	0.25	0.297	0.28	0.240
IVRT/R-R interval	0.47	0.035	0.25	0.295	0.61	0.004
PV DI-wave velocity (cm/s)	-0.11	0.642	0.03	0.899	-0.07	0.767
PV VTID (cm)	-0.14	0.557	-0.28	0.237	-0.20	0.403
IVST (mm)	0.66	0.002	0.78	<0.0001	0.79	<0.0001
LVPWT (mm)	0.62	0.004	0.66	0.001	0.59	0.045
RWT	0.68	0.001	0.78	<0.0001	0.82	<0.0001
LA	0.31	0.186	0.53	0.016	0.52	0.018
LVEDV (μl)	-0.55	0.012	-0.67	0.001	-0.79	<0.0001
LVESV (μl)	-0.56	0.009	-0.68	<0.001	-0.79	<0.0001

Values are presented as the mean ± SD. Abbreviations are the same as described above in Table 2.