CHANNELS
2020, VOL. 14, NO. 1, 231–245
https://doi.org/10.1080/19336950.2020.1794420

Taylor & Francis
Taylor & Francis Group

RESEARCH PAPER

OPEN ACCESS Check for updates

# A low voltage activated $Ca^{2+}$ current found in a subset of human ventricular myocytes

Xin Zhang[a,b], Yijia Li[b], Xiaoying Zhang [b], Valentino Piacentino III[b,c], David M. Harris[b,d], Remus Berretta[b], Kenneth B. Margulies[b,e], Steven R. Houser[b], and Xiongwen Chen[b]

[a]Department of Infection Diseases The First Affiliated Hospital of China Medical University, Shenyang China; [b]Department of Physiology and Cardiovascular Research Center, Temple University Lewis Katz School of Medicine, Philadelphia, PA, USA; [c]Department Grand Strand Surgical Care, Grand Strand Regional Medical Center, Myrtle Beach, SC; [d]College of Medicine, University of Central Florida, Orlando, Florida, USA; [e]Department of Medicine, University of Pennsylvania, Philadelphia, PA, USA

**ABSTRACT**

Low voltage activated ($I_{Ca\text{-}LVA}$) calcium currents including Cav1.3 and T-type calcium current ($I_{Ca\text{-}T}$) have not been reported in adult human left ventricular myocytes (HLVMs). We tried to examine their existence and possible correlation with etiology and patient characteristics in a big number of human LVMs isolated from explanted terminally failing (F) hearts, failing hearts with left ventricular assist device (F-LVAD) and nonfailing (NF) human hearts. LVA ($I_{Ca\text{-}LVA}$) was determined by subtracting L-type $Ca^{2+}$ current ($I_{Ca\text{-}L}$) recorded with the holding potential of −50 mV from total $Ca^{2+}$ current recorded with the holding potential of −90 mV or −70 mV. $I_{Ca\text{-}LVA}$ was further tested with its sensitivity to 100 μM $CdCl_2$ and tetrodotoxin. Three HLVMs (3 of 137 FHLVMs) from 2 (N = 30 hearts) failing human hearts, of which one was idiopathic and the other was due to primary pulmonary hypertension, were found with $I_{Ca\text{-}LVA}$. $I_{Ca\text{-}LVA}$ in one FHLVM was not sensitive to 100 μM $CdCl_2$ while $I_{Ca\text{-}LVA}$ in another two FHLVMs was not sensitive to tetrodotoxin. It peaked at the voltage of −40~-20 mV and had a time-dependent decay faster than $I_{Ca\text{-}L}$ but slower than sodium current ($I_{Na}$). $I_{Ca\text{-}LVA}$ was not found in any HLVMs from NF (75 HLVMs from 17 hearts) or F-LVAD hearts (82 HLVMs from 18 hearts) but a statistically significant correlation could not be established. In conclusion, $I_{Ca\text{-}LVA}$ was detected in some HLVMs of a small portion of human hearts that happened to be nonischemic failing hearts.

**ARTICLE HISTORY**
Received 14 March 2020
Revised 18 June 2020
Accepted 22 June 2020

**KEYWORDS**
Heart failure; low voltage activated $Ca^{2+}$ current; Cav1.3; T-type calcium channel; ventricular myocytes; left ventricular assist device

## Introduction

Calcium ($Ca^{2+}$) influx through calcium channels plays important roles in the heart. It not only mediates excitation-contraction coupling, but also regulates many other processes such as transcription, myocyte death and hypertrophy [1]. Two types of calcium channels, high-voltage activated (mainly Cav1.2 L-type calcium channel, LTCC) and low voltage activated calcium channels ($I_{Ca\text{-}LVA}$, Cav1.3 L-type and Cav3 T-type calcium channels, TTCCs), are present at the gene expression and functional levels in cardiomyocytes [2,3]. Three types of TTCCs, Cav3.1 (a1 G), Cav3.2 (a1 H) and Cav3.3 (a1I), are encoded by three genes, CACNA1 G, CACNA1 H, and CACNA1I, respectively. The former two are the ones expressed in the heart [2,3]. As with Cav3.1 and Cav3.2, Cav1.3 is normally present in pacemaker tissues participating cardiac rhythm generation and atria but has not been reported in mammalian ventricular myocytes [4].

It has been reported that the expression of TTCCs in cardiomyocytes depends on species, developmental stages, cardiac regions, disease states and cell cycles of cardiomyocytes [2,5]. TTCCs are ubiquitously expressed in cardiomyocytes of all chambers in mammalian fetal hearts and may participate in fetal myocyte excitation-contraction coupling [6]. In fetal mouse hearts, the expression of Cav3.1 and Cav3.2 are temporally and spatially different. For example, in mice, Cav3.1 appears at E8.5 in all myocytes and outflow tract cells while Cav3.2 mRNA

**CONTACT** Steven R. Houser srhouser@temple.edu; Xiongwen Chen xchen001@temple.edu

**New and Noteworthy**

A low voltage activated $Ca^{2+}$ current ($I_{Ca\text{-}LVA}$), probably including Cav3 (T-type) and Cav1.3 mediated L-type $Ca^{2+}$ currents, was found in some human left ventricular myocytes from failing hearts. This current was inactivated by holding the cell at −50mV, not sensitive to 100μM $CdCl_2$ or tetrodotoxin, peaked at around −30mV and decayed at rates slower than $Na^+$ current but faster than Cav1.2 mediated $I_{Ca\text{-}L}$.

expresses at a later stage and has the highest expression in the atrioventricular canal [7]. In human ventricles, Cav3.2 mRNA is present in fetal hearts and decreases during heart development [8]. After birth, TTCC expression remains in the automatic tissues and the conduction system but disappears in other regions of the heart like the ventricles. As TTCCs remain in the sinoatrial and atrioventricular node and the conduction system, it has been proposed that the major function of TTCCs in adult hearts is to initiate cardiac rhythm and regulate cardiac conduction [2]. Recently, we showed that TTCCs play an important role in heart rate regulation by the sympathetic/β-adrenergic system [9]. $I_{Ca\text{-}T}$ expression in rat atrial myocytes decreases during postnatal development [10,11]. In general TTCCs are absent in adult ventricular myocytes (VMs) of mammals with the exception of a few species including guinea pigs [12,13]. However, thus far, there has been no $I_{Ca\text{-}T}$ reported in adult human ventricular myocytes [14].

While TTCCs participates in excitation-contraction coupling in fetal ventricular myocytes [6], their role in adult ventricular myocytes is still elusive. $I_{Ca\text{-}T}$ in adult guinea pig ventricular myocytes participates in excitation-contraction coupling but the efficiency is low [15]. With cardiomyocyte specific transgenic expression of Cav3.1 in mice, we showed that T-type $Ca^{2+}$ current is ineffective to load the SR, neither does it induce $Ca^{2+}$ release from the SR [16]. Interestingly, $I_{Ca\text{-}T}$ expression was found to be associated with the S phase of cell cycle in cultured neonatal rat ventricular myocytes [17]. We have reported that TTCCs were expressed in high percentage of mononucleated ventricular myocytes of adolescent feline hearts, which may be related to their potential of proliferation [18]. $I_{Ca\text{-}T}$ has been reported to reappear in ventricular myocytes from diseased hearts induced by pressure-overload, post myocardial infarction, and dystrophin deficiency [2,19]. It is not entirely clear the significance of reappearance of TTCCs in stressed hearts. Cav3.2 may provide $Ca^{2+}$ signal for cardiac hypertrophy [20] while Cav3.1 mediated $Ca^{2+}$ influx is antihypertrophic via the NO/cGMP/PKG signaling pathway [21].

Cav1.3 belongs to L-type $Ca^{2+}$ channels and is predominantly expressed in pacemaking tissues and weakly in the atria. Its existence in human ventricles is generally considered absent [4,22] but Lu et al. detected Cav1.3 proteins in human ventricular tissue [23]. Compared to Cav1.2 channels, Cav1.3 channels are activated at quite low voltages overlapping the range of activation voltages for Cav3, inactivate more slowly and less sensitive to dihydropyridine LTCC blockers than Cav1.2 [24]. Yet, Cav1.3 current is sensitive to the blockade by 100 μM $CdCl_2$, as is Cav1.2 current [25].

It has been reported that there is another calcium permeable channel activated at low voltage in sodium-free bath solution, the TTX-sensitive sodium channel, in cardiac myocytes. The current through this type of channel was termed as TTX-sensitive calcium current ($I_{Ca(TTX)}$). $I_{Ca(TTX)}$ was first reported in rat ventricular myocytes [26] and also found in human atrial myocytes [27]. However, its existence has been controversial [28].

This study is to determine if there is low-voltage activated calcium currents ($I_{Ca\text{-}LVA}$) in human ventricular myocytes by examining a large number of failing and nonfailing HLVMs and to characterize this low-voltage activated calcium current. We found a small portion of failing human hearts had some LVMs expressing a low-voltage activated, partially or fully inactivated by the holding potential of −50 mV but TTX-insensitive current. This current was not detected in HLVMs from nonfailing hearts or failing hearts with LVAD support.

## Methods

### *Myocyte isolation*

Human left ventricular myocytes (HLVMs) were isolated from the midwall of the LV free wall of 17 nonfailing (NF), 30 failing (F) and 18 failing human hearts with left ventricular assist device (LVAD) support as described previously [29] and used within 12 hours after isolation. Failing and LVAD–supported failing human hearts were obtained from the Temple Cardiac Transplant Team at the time of cardiac transplantation. Nonfailing hearts were donor hearts unsuitable for transplantation. Our protocol was approved by Temple University Institutional Review Board. Patient characteristics were presented in **Table 1** with detailed information in **Table 3**.

### Cav3.2 transgenic mouse models and ventricular myocyte isolation

To compare $I_{Ca\text{-}T}$ expressed in native cardiomyocytes, a mouse model with cardiac-specific (controlled by α-MHC promoter) and conditional (tet-off, controlled by doxycycline, DOX) overexpression of human Cav3.2 (Genebank ID: NM_021098) was established with the bitransgenic system developed by Sanbe A et al. [30] and gifted by the Molkentin group at University of Cincinnati. Ventricular myocytes (VMs) was isolated as described previously [31]. All animal work was approved by Institutional Animal Use and Care Committee of Temple University.

### $Ca^{2\pm}$ current measurements

Whole cell $Ca^{2+}$ currents ($I_{Ca\text{-}L}$) were measured in $Na^+$- and $K^+$-free solutions at 37°C using techniques described in detail previously [9,16,18,21,32]. Briefly, myocytes were added into a water-heated chamber mounted on an inverted microscope (Nikon, Japan) and were initially perfused with a physiological salt solution containing (in mmol/L): NaCl 150, KCl 5.4, $CaCl_2$ 1, $MgCl_2$ 1.2, glucose 10, sodium pyruvate 2, and HEPES 5 (pH7.4) at 37°C. Low resistance (1–3 MΩ) micropipettes filled with a solution containing (in mmol/L) Cs-aspartate 130, NMDG 10, TEA-Cl 20, Tris-ATP 2.5, Tris-GTP 0.05, $MgCl_2$ 1, EGTA 10, pH 7.2, were used for whole cell voltage clamp experiments. The overall junction potential was around −10 mV and not corrected in the analysis. Once a gigaohm seal was obtained, the membrane was ruptured for dialysis while the voltage was set at −70 mV. After myocytes were dialyzed with the internal solution and perfused with the physiological salt solution for 10 minutes, the bath solution was switched to a $Na^+$ and $K^+$ free bath solution containing (in mmol/L): NMDG 150, $CaCl_2$ 2, CsCl 5.4, $MgCl_2$ 1.2, Glucose 10, HEPES 5, 4-AP (4-aminopyridine) 2 (pH = 7.4). Three minutes of high-flow (5 mL/min) washing with the $Na^+$ and $K^+$ free bath solution was done to completely switch out $Na^+$ containing physiological solution. Discontinuous switching voltage clamp was achieved with an Axoclamp-2B (Axon Instruments) amplifier. Vm and Im output bandwidth was set at 10 KHz. The switching rate was set at least 7 kHz. The gain was set at least 3 (normally 5–12) to quickly charge and discharge the membrane and to minimize the effect of capacitance charging/discharging currents on recorded currents (mostly achieving 90% voltage control within 2 ms). The Clampex 8 software (Axon Instrument) was used to control the amplifier and to acquire data with a Digidata 1200 (Axon Instruments) analog-to-digital converter. To define the total $I_{Ca}$–voltage relationship, the membrane potential was held at – 70 mV or −90 mV and then depolarized in a 10 mV-increment. The existence of $I_{Ca\text{-}LVA}$ was examined retro-respectively and the correlation of its existence to cell and patient characteristics were tested. In one myocyte with $I_{Ca\text{-}LVA}$ identified, $I_{Ca}$-voltage relationships were examined with holding potentials at both −90 mV and −50 mV in a 10-mV increment in the presence of 50 μM TTX or 100 μM $Cd^{2+}$. Currents were

**Table 1.** Patient characteristics.

| Patient | Etiology | Male/ Female | Age (y) | CHF Duration (months) | Duration of LVAD (days) | LVEF (%) |
|---|---|---|---|---|---|---|
| NF (*N = 17*) | - | 9/8 | 60.2 ± 3.9 | 0 | - | 55.2 ± 1.9 |
| F (*N = 30*) | Idiopathic 7<br>Ischemic 18<br>Hypertension 2<br>Valvular 2<br>Dilated cardiomyopathy 1 | 22/8 | 53.5 ± 2.0 | 62.1 ± 15.1 | - | 13.6 ± 2.0*** |
| LVAD (*N = 18*) | Idiopathic 7<br>Ischemic 8<br>Hypertension 1<br>Postpartum 1<br>Toxic 1 | 10/8 | 52.9 ± 2.5 | 69.7 ± 13.6 | 194 ± 59.9 | 14.7 ± 3.9***<br>(pre-LVAD value) |

Data from individual patients, including medications is presented in the on-line supplement. ***: $p < 0.001$ versus NF (one-way ANOVA).

recorded and analyzed offline with Clampfit 8. A subtraction of the average of the tail current of last two milliseconds of the depolarization from the peak was done before the construction of $I_{Ca}$-voltage relationships. The decay of $I_{Ca\text{-}L}$ and $I_{Ca\text{-}LVA}$ currents was fitted with a double- or single-exponential decay function built in Clampfit 8, respectively. Minimal sum of squared errors and maximal correlation coefficient (>0.90) were used to determine the goodness of the fitting. Time constants (fast time constants ($\tau_f$) and slow time constants ($\tau_s$) for the double-exponential decay function, a single time constant ($\tau$) for the single-exponential decay function) were obtained from the fitting.

### $I_{Na}$ recording and analysis

To simulate potential residual $Na^+$ current and contaminating $I_{Na}$, we added 5 mM $Na^+$ in the bath solution used for $I_{Ca}$ recording (composition in mM: NaCl 5, NMDG 150, $CaCl_2$ 2, CsCl 5.4, $MgCl_2$ 1.2, Glucose 10, HEPES 5, 4-AP 2 (pH = 7.4). In a HLVM without $I_{Ca\text{-}LVA}$, $I_{Na}$ was recorded with $I_{Ca\text{-}L}$ when the myocyte was held at the $V_h$ of −90 mV and depolarized with 400 ms test pulses in a 10 mV increment while $I_{Ca\text{-}L}$ was recorded with $I_{Na}$ inactivated by the $V_h$ = −50 mV. $I_{Na}$ was obtained by subtracting $I_{Ca\text{-}L}$ recorded at $V_h$ = −50 mV from $I_{Ca\text{-}L}$+ $I_{Na}$ recorded at $V_h$ = −90 mV. The current-voltage (I–V) relationship of $I_{Na}$ was constructed by plotting peak $I_{Na}$ versus test potentials. The inactivation of $I_{Na}$ was fit with a single exponential decay function built in Clampfit 8 from the peak to 200 ms thereafter. Minimal sum of squared errors and maximal correlation coefficient (>0.90) were used to determine the goodness of the fitting.

### Statistics

Data in the text and tables are reported as mean ±SEM. One-way ANOVA was used to detect difference between patient parameters of the nonfailing, failing, and LVAD-failing groups with SAS 9.0 (SAS Institute Inc., Cary, NC). A $p$ value of 0.05 was considered significant. Contingency table was used to determine if the occurrence of $I_{Ca\text{-}LVA}$ in failing vs. nonfailing human hearts or ventricular myocytes was significantly different.

## Results

### $I_{Ca\text{-}LVA}$ existence and its association with etiologies

We totally examined 75 nonfailing LVMs from 17 NF hearts, 137 LVMs from 30 F hearts and 82 LVMs from 18 LVAD-support failing hearts for the existence of $I_{Ca\text{-}LVA}$ currents. Of these failing hearts, 7 were idiopathic, 18 ischemic, 2 hypertensive, 2 related to valvular diseases, and 1 with dilated cardiopathy. Of these LVAD-supported failing hearts, 7 were idiopathic, 8 ischemic, 1 hypertensive, 1 with postpartum heart failure, and 1 due to drug toxic. Three LVMs with $I_{Ca\text{-}LVA}$ were all found in failing hearts (2 out of 30 hearts, 6.7%) but in none of nonfailing (0 out 18 hearts) and failing with LVAD-support (0 out of 18) hearts (**Table 2**). One LVM with $I_{Ca\text{-}LVA}$ was found among 7 examined LVMs (14.3%) from a failing heart with hypertrophic cardiomyopathy (heart #2 in "HF Patients" table of Table 3, female). Two LVMs out of 8 examined LVMs (25%) from a failing heart with primary pulmonary hypertension (heart #27 in "HF Patients" table of Table 3, male) were found with $I_{Ca\text{-}LVA}$.

### $I_{Ca\text{-}LVA}$ current in a failing HLVM is $Cd2^{\pm}$ insensitive but inactivated by a holding voltage of −50 mV

Figure 1 shows $I_{Ca}$ recorded in a failing human ventricular myocyte. Total $I_{Ca}$ recorded at −50~-30 mV from the holding potential of −70 mV had two peaks/components (Figure 1a), indicating the co-existence of significant low-voltage ($I_{Ca\text{-}LVA}$) and high-voltage (L-type) $Ca^{2+}$ currents in one

**Table 2.** Percentage of hearts and HLVMs with $I_{Ca\text{-}LVA}$ current, in NF, F and LVAD hearts and HLVMs.

| | Total Heart Number | Hearts with $I_{Ca\text{-}LVA}$ | % of hearts with $I_{Ca\text{-}LVA}$ | Total Cells Examined | Cells with $I_{Ca\text{-}LVA}$ | Percentage of HLVMs with $I_{Ca\text{-}LVA}$ |
|---|---|---|---|---|---|---|
| Nonfailing | 17 | 0 | 0% | 75 | 0 | 0% |
| Failing | 30 | 2 | 6.7% | 137 | 3 | 2.18% |
| Failing-LVAD | 18 | 0 | 0% | 82 | 0 | 0% |

**Table 3.** Characteristics of nonfailing donors and failing and failing-LVAD patients.

| Patient | Age (y) | Sex | Body Weight (kg) | Heart Weight (g) | LVEF (%) | Medication |
|---|---|---|---|---|---|---|
| NF Donors (N = 17) | | | | | | |
| 1 | 58 | f | 55 | 378 | 60 | Db,BB,N |
| 2 | 16 | f | 52 | 373 | 52.5 | Db,M |
| 3 | 66 | m | 70 | 518 | 60 | N |
| 4 | 79 | m | 77 | 583 | - | ACEI,Du |
| 5 | 64 | f | 48 | 303 | 55 | - |
| 6 | 65 | m | 88 | 534 | 57.5 | - |
| 7 | 62 | f | 64 | 369 | 45 | Dg |
| 8 | 40 | m | 85 | - | 50 | BB |
| 9 | 72 | m | 77 | 495 | - | ACEI,BB,CaB |
| 10 | 68 | m | 66 | 471 | - | BB,AB |
| 11 | 59 | m | 113 | 530 | 55 | BB,AB |
| 12 | 51 | f | 81 | 378 | 62.5 | ACEI,BB,AB |
| 13 | 76 | m | 70 | 365 | - | - |
| 14 | 43 | f | 70 | 335 | - | - |
| 15 | 61 | m | - | 472 | - | - |
| 16 | 72 | f | - | 374 | - | - |
| 17 | 72 | f | - | 411 | - | - |
| Mean±SEM | 60 ± 4 | | 73 ± 4 | 431 ± 20 | 55 ± 1 | |

| Patient | Age (y) | Sex | Body Weight (kg) | Heart Weight (g) | CHF Duration (mo) | Etiology | CI (L/min) | LVEF (%) | Medication |
|---|---|---|---|---|---|---|---|---|---|
| HF patients (N = 30) | | | | | | | | | |
| 1 | 52 | f | 92 | 762 | 144 | Idio | 2.57 | 22.5 | M,ACEI,ANGI,Dg,Du,Am |
| 2 | 30 | f | 59 | 429 | - | Hypertrophic | - | - | M,BB,Dg,Du |
| 3 | 62 | m | 91 | 617 | - | Isch | - | 17.5 | ACEI,BB,CaB,N,Dg,Du,Am |
| 4 | 20 | m | 64 | 377 | 2 | Idio | 3.6 | 7.5 | Db,ANGI,Dg,Du |
| 5 | 62 | m | 67 | 417 | - | Isch | 4.51 | 17.5 | M,ACEI,N,Dg,Du |
| 6 | 48 | f | 66 | - | - | Val | 2.6 | 10 | M,ACEI,ANGI,N,Du,Am |
| 7 | 62 | m | 86 | 668 | - | Isch | 4.6 | 17.5 | M,ANGI,BB,N,Hy,Du |
| 8 | 46 | m | 77 | 451 | 77 | Idio | 3.1 | 5 | M,ACEI,ANGI,CaB,Dg,Du |
| 9 | 45 | m | 74 | 488 | - | Isch | 1.4 | 10 | Db,M,ACEI,ANGI,N,Dg,Du,Am |
| 10 | 65 | m | 79 | 604 | 57 | Isch | 3.05 | 7.5 | Db,M,ANGI,Dg,Am |
| 11 | 59 | f | 68 | 527 | - | Isch | 2.2 | 5 | Db,M,ACEI,N,Dg |
| 12 | 64 | m | 78 | 630 | 20 | Isch | 3 | 12.5 | Db,M,ACEI,N,Dg |
| 13 | 62 | m | 80 | 599 | 43 | Isch | 3 | 12.5 | Db,M,ANGI,CaB,N,Hy,Dg,Du |
| 14 | 37 | m | 93 | 575 | - | Idio | 1.5 | 12.5 | Db,M,ACEI,CaB,N,Hy,Du,Am |
| 15 | 58 | m | 53 | 493 | - | Isch | - | 12.5 | Db,M,ACEI,Am |
| 16 | 61 | m | 76 | 470 | - | Isch | 2.1 | 10 | Db,M,ACEI,ANGI,N,Dg,Du,Am |
| 17 | - | - | - | - | - | Htn | - | - | - |
| 18 | 57 | m | 69 | 587 | - | Isch | 2 | 7.5 | M,ACEI,ANGI,BB,N,Dg,Du |
| 19 | 52 | m | 111 | 553 | - | Isch | 2.12 | 12.5 | M,ACEI,N,Dg,Du,Am |
| 20 | 62 | f | 90 | 839 | 132 | Isch | 3.1 | 12.5 | Db,Du |
| 21 | 61 | f | 76 | 374 | - | Isch | 4.75 | 57.5 | BB,CaB,N,Du |
| 22 | 50 | m | 73 | 366 | 36 | Idio | - | 7.5 | ACEI,BB,Dg,Du |
| 23 | 57 | m | - | 611 | - | Idio | - | 7.5 | - |
| 24 | 47 | f | 59 | 337 | 72 | DCM | 1.9 | 15 | Db,M,ACEI,BB,Du |
| 25 | 56 | m | 72 | 1034 | 4 | Isch | - | 7.5 | Db,M,ACEI,Dg,Du,Am |
| 26 | 58 | m | 85 | 761 | - | Valv | 2.28 | 7.5 | M,N,Hy,Dg,Du,Am |

(*Continued*)

**Table 3.** (Continued).

| Patient | Age (y) | | Sex | Body Weight (kg) | Heart Weight (g) | | LVEF (%) | Medication |
|---|---|---|---|---|---|---|---|---|
| NF Donors (N = 17) | | | | | | | | |
| 27 | 41 | m | - | 550 | - | PPHTn | 1.7 | 30 | Db,Am |
| 28 | 64 | m | 77 | 492 | 96 | Isch | - | 10 | Db,M,ACEI,N,Dg,Du |
| 29 | 59 | m | 100 | - | - | Isch | - | 12.5 | ACEI,BB,N,Du,Am |
| 30 | 55 | m | - | 602 | - | Isch | - | 12.5 | ACEI,BB,N,Hy,Du |
| Mean±SEM | 54 ± 2 | | 78 ± 2 | 563 ± 29 | 62 ± 9 | | 3 ± 0 | 14 ± 2 | |

| Patient | Duration of LVAD (days) | Age (y) | Sex | Body Weight (kg) | Heart Weight (g) | CHF Duration (month) | Etiology | Pre-LVAD CI (L/min) | LVEF (%) | Medication |
|---|---|---|---|---|---|---|---|---|---|---|
| LVAD patients (N = 18) | | | | | | | | | | |
| 1 | - | 60 | m | 79 | 26 | 136 | Isch | 1.8 | 10 | - |
| 2 | 28 | 48 | m | 68 | 22 | 172 | Isch | 2.3 | 7.5 | - |
| 3 | 83 | 59 | m | 74 | 24 | 60 | Idio | 1.8 | 7.5 | ANGI,Am |
| 4 | 540 | 66 | f | 72 | 31 | - | Isch | 1.9 | 35 | Db,BB,AB,CaB,N |
| 5 | 130 | 54 | m | 80 | 24 | 105 | Idio | 3.2 | 7.5 | - |
| 6 | 160 | 56 | f | 63 | 26 | 57 | Isch | 1.5 | 7.5 | ANGI,AB,N,Am |
| 7 | 8 | 41 | m | 93 | 27 | 3 | Isch | - | 15 | M,BB,N |
| 8 | 23 | 54 | m | 79 | 24 | 7 | Idio | 2 | - | N,Dg,Du,Am |
| 9 | - | 34 | f | 66 | 27 | 108 | PP | - | 7.5 | ACEI,ANGI,BB,Dg,Du |
| 10 | 164 | 53 | f | 65 | 26 | - | Htn | 1 | 7.5 | M,Du |
| 11 | 6 | 39 | m | 102 | 37 | 58 | Idio | 1.7 | 10 | - |
| 12 | - | 63 | m | 76 | 25 | 54 | Isch | 2.58 | 12.5 | M,ACEI,Dg |
| 13 | 390 | 31 | f | 70 | 24 | 1 | Idio | 2.24 | 42.5 | Dg |
| 14 | 4 | 56 | m | 89 | 33 | 94 | Isch | 3.2 | 12.5 | M,Dg |
| 15 | 473 | 53 | f | 73 | 26 | 87 | Idio | - | 5 | CaB |
| 16 | 508 | 57 | m | 81 | 28 | 94 | Isch | 2.2 | 7.5 | BB,AB,Du |
| 17 | - | 66 | f | 75 | 25 | - | Idio | - | 10 | Dg,Du |
| 18 | - | 63 | f | - | - | 9 | Toxic | - | 7.5 | CaB,Am |
| Mean ±SEM | 194 ± 49 | 53 ± 2 | | 77 ± 2 | 27 ± 1 | 70 ± 12 | | 2 ± 0 | 15 ± 3 | |

AB, α-adrenergic blocker; ACEII, angiotensin-converting enzyme inhibitor; Am, amiodarone; ANGI, angiotensin II inhibitor (angiotensin receptor blocker); BB, β-adrenergic blocker; CaB, calcium channel blocker; CHF, congestive heart failure; CI, cardiac index; Db, dobutamine; DCM, dilated cardiomyopathy; Dg, digoxin; Dop, Dopamine; Du, diuretic; Htn, hypertension; Hy, Hydralazine; Idio, Idiopathic cardiomyopathy; Isch, ischemic cardiomyopathy; LVEF, LV ejection fraction; M, Milrinone; N, nitrate; PPHTn, Primary pulmonary hypertension; PP, postpartum; -, unknown; Valv, valvular cardiomyopathy.

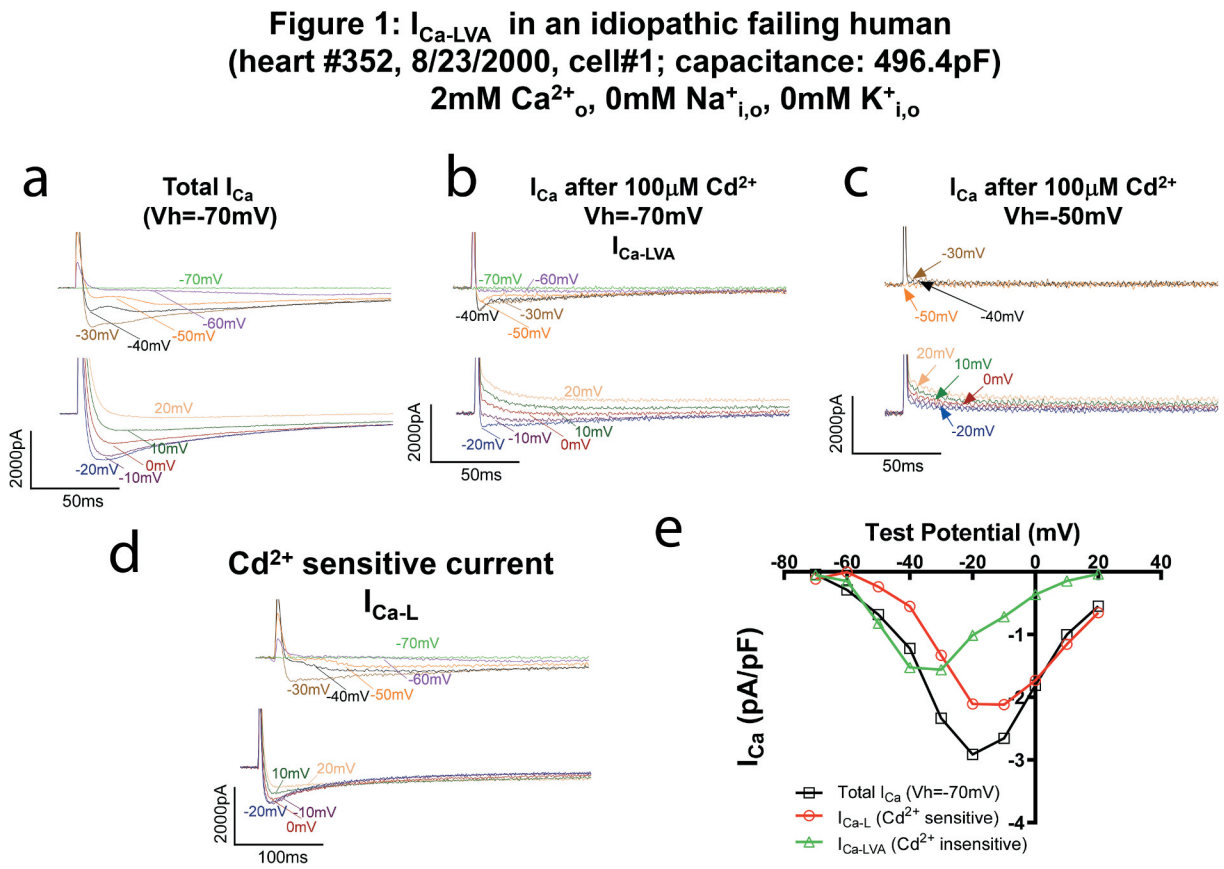


**Figure 1.** $I_{Ca\text{-}LVA}$ in a failing HLVM from a hypertrophic cardiomyopathy heart is insensitive to 100 μM $CdCl_2$ but inactivated by the holding potential of −50 mV. (a–d). Current traces at different test voltages (in 10 mV increment from the holding potential, not adjusted for junctional potential) of total $I_{Ca}$ recorded from the holding potential of −70 mV (a), of residual $I_{Ca}$ recorded with the holding potential of −70 mV after the application of 100uM $Cd^{2+}$ (b, presumably $I_{Ca\text{-}T}$), of currents recorded with the holding potential of −50 mV after the application of 100uM $Cd^{2+}$ (c), and of $Cd^{2+}$-sensitive currents (differential current obtained by subtracting total $I_{Ca}$ from $Cd^{2+}$ insensitive current, presumably $I_{Ca\text{-}L}$, d). (e). The current (i)-Voltage (v) relationships of total $I_{Ca}$, $Cd^{2+}$ sensitive $Ca^{2+}$ current ($I_{Ca\text{-}L}$) and $Cd^{2+}$-insensitive but holding potential sensitive current (likely $I_{Ca\text{-}T}$).

cell. Since L-type $Ca^{2+}$ current ($I_{Ca\text{-}L}$) is sensitive but $I_{Ca\text{-}T}$ is insensitive to 100 μM $Cd^{2+}$, we tested the sensitivity of these two $Ca^{2+}$ current components to $Cd^{2+}$. We found that there was $Cd^{2+}$-insensitive $Ca^{2+}$ current (Figure 1b) that peaked at −30 mV and inactivated by holding the cell at −50 mV (Figure 1c). The decay of these currents was faster than the $Cd^{2+}$-sensitive current (Figure 1d). In contrast, the $Cd^{2+}$ sensitive current lost the first hump in the current traces (Figure 1d). It had a threshold for activation at −50 mV and peaked at −10 mV (Figure 1e), which was more negative than the usual activation threshold (~-20 mV) and peak (0–10 mV) voltages in normal human and animal ventricular myocytes. This negative activation and peak could be due to the hyperphosphorylation of LTCC in failing myocytes [29] or a combined Cav1.3 and Cav1.2 currents. The peak amplitude of presumed $I_{Ca\text{-}T}$ was substantial even it could be underestimated because the holding potential of −70 mV could inactivate a large fraction of $I_{Ca\text{-}T}$.

In contrast, in a failing HLVM without $I_{Ca\text{-}LVA}$ as shown in Figure 2, 100 μM $Cd^{2+}$ completely abolished all $Ca^{2+}$ currents recorded with a Vh of −90 mV (**Figure 2**a,c). There was no difference between total $I_{Ca}$ measured with the $V_h$ of −90 mV (Figure 2a), the L-type $Ca^{2+}$ current measured with the $V_h$ of −50 mV (Figure 2b) and the $Cd^{2+}$-sensitive currents (Figure 2e). The I–V relationships of total $I_{Ca}$, $I_{Ca\text{-}L}$ and the $Cd^{2+}$ sensitive currents were overlapping very well (figure 2f). The threshold for activation was −20 mV and the density was greater than that of the failing myocyte

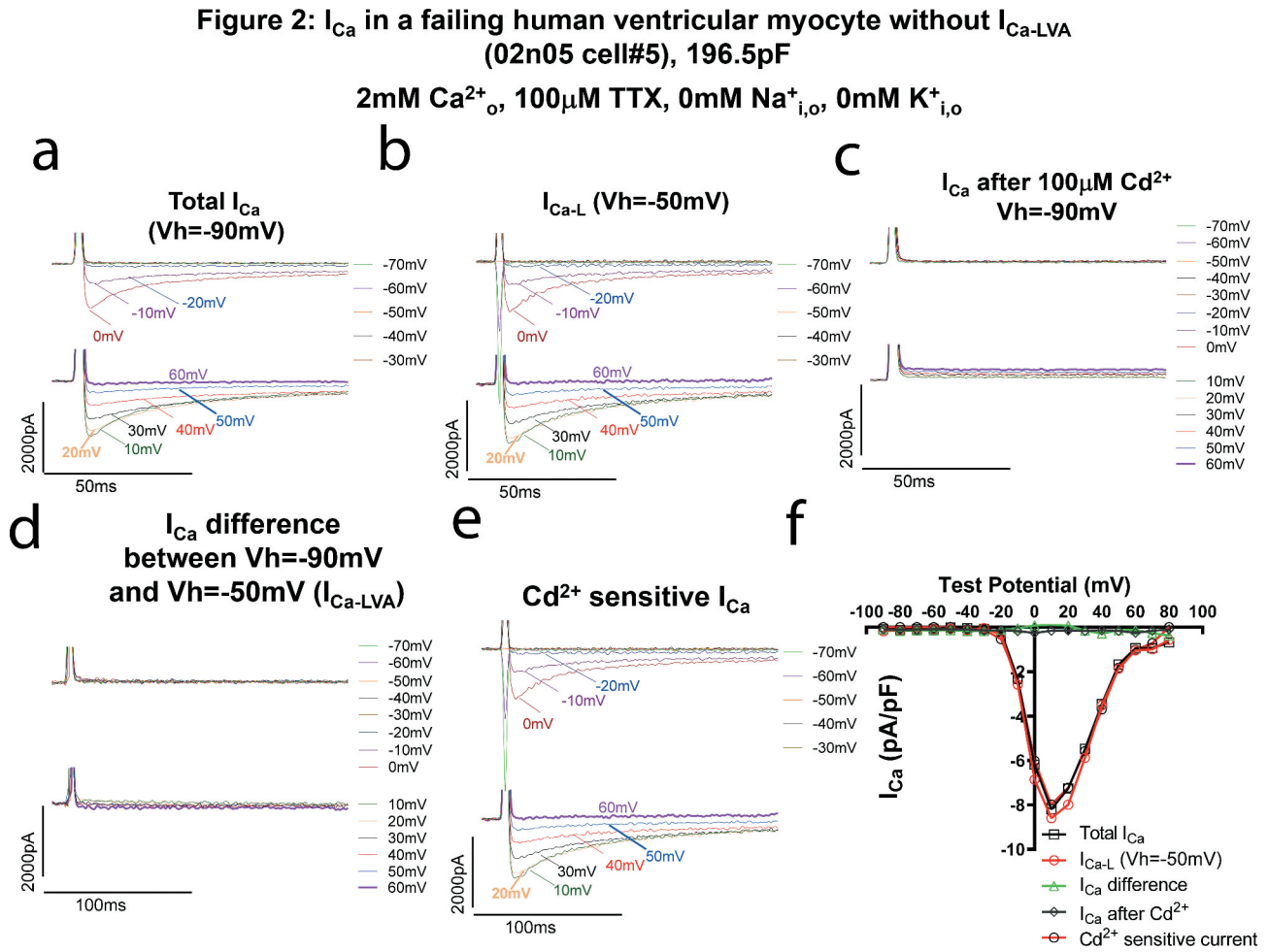


**Figure 2.** $Ca^{2+}$ currents in a failing HLVM without $I_{Ca-LVA}$. All $I_{Ca}$ was abolished by 100 μM $CdCl_2$. (a–d). Current traces at different test voltages (in a 10 mV-increment from the holding potential) of total $I_{Ca}$ recorded from the holding potential of −90 mV (not adjusted for junctional potential which was about −10 mV) (a), of $I_{Ca-L}$ recorded from the holding potential of −50 mV (b), of currents recorded from the holding potential of −50 mV after the application of 100uM $Cd^{2+}$ (c), of differential currents between total $I_{Ca}$ and $I_{Ca-L}$ (d), and of $Cd^{2+}$-sensitive currents (difference current obtained by subtracting total $I_{Ca}$ from $Cd^{2+}$ insensitive current, presumably $I_{Ca-L}$, (e). In B, please note that the fast downward currents recorded were capacitance transients (currents charging and discharging the membrane) when we hyperpolarized the cell from −50 mV to −90 mV~-60 mV. (**f**). Current (i)-Voltage (v) relationships of total $I_{Ca}$, $I_{Ca}$ recorded with the holding potential of −50 mV, differential currents between total $I_{Ca}$ and $I_{Ca-L}$, $Cd^{2+}$-insensitive and $Cd^{2+}$ sensitive currents. The I–V curves of total $I_{Ca}$, $I_{Ca}$ recorded with the $V_h$ of −50 mV and $Cd^{2+}$ sensitive currents were overlapping.

in Figure 1, suggesting this $I_{Ca-L}$ was more likely mediated by Cav1.2 only while $I_{Ca-L}$ in Figure 1 could contain both Cav1.2 and Cav1.3 currents that were down-regulated by heart failure.

### *$I_{Ca-LVA}$ current in 2 failing HLVMs is not sensitive to TTX and inactivates faster than $I_{Ca-L}$*

We found another cell with $I_{Ca-LVA}$ like current which appeared at the test potential of −40 mV from the holding potential of −90 mV in the presence of tetrodotoxin (TTX, 100 μM). Total $I_{Ca}$, $I_{Ca-L}$ ($V_h$ = −50 mV) and $I_{Ca-LVA}$ ($I_{Ca}$ difference between total $I_{Ca}$ and $I_{Ca-L}$) recorded in this TTX containing solution were shown in **Figure 3**a–c. While $I_{Ca-L}$ recorded at Vh = −50 mV could be better fit with a double exponential decay equation, $I_{Ca-LVA}$ was well fit with a single exponential decay equation. The slow and fast time constants ($\tau_s$ and $\tau_f$) of $I_{Ca-L}$ and time constant ($\tau$) of $I_{Ca-LVA}$ were shown Figure 3d and e, respectively. The time constants ($\tau$) for $I_{Ca-LVA}$ were smaller than the slow time constants ($\tau_s$) of $I_{Ca-L}$ and similar to fast $\tau$ values of $I_{Ca-L}$, consistent with a previous report [33].

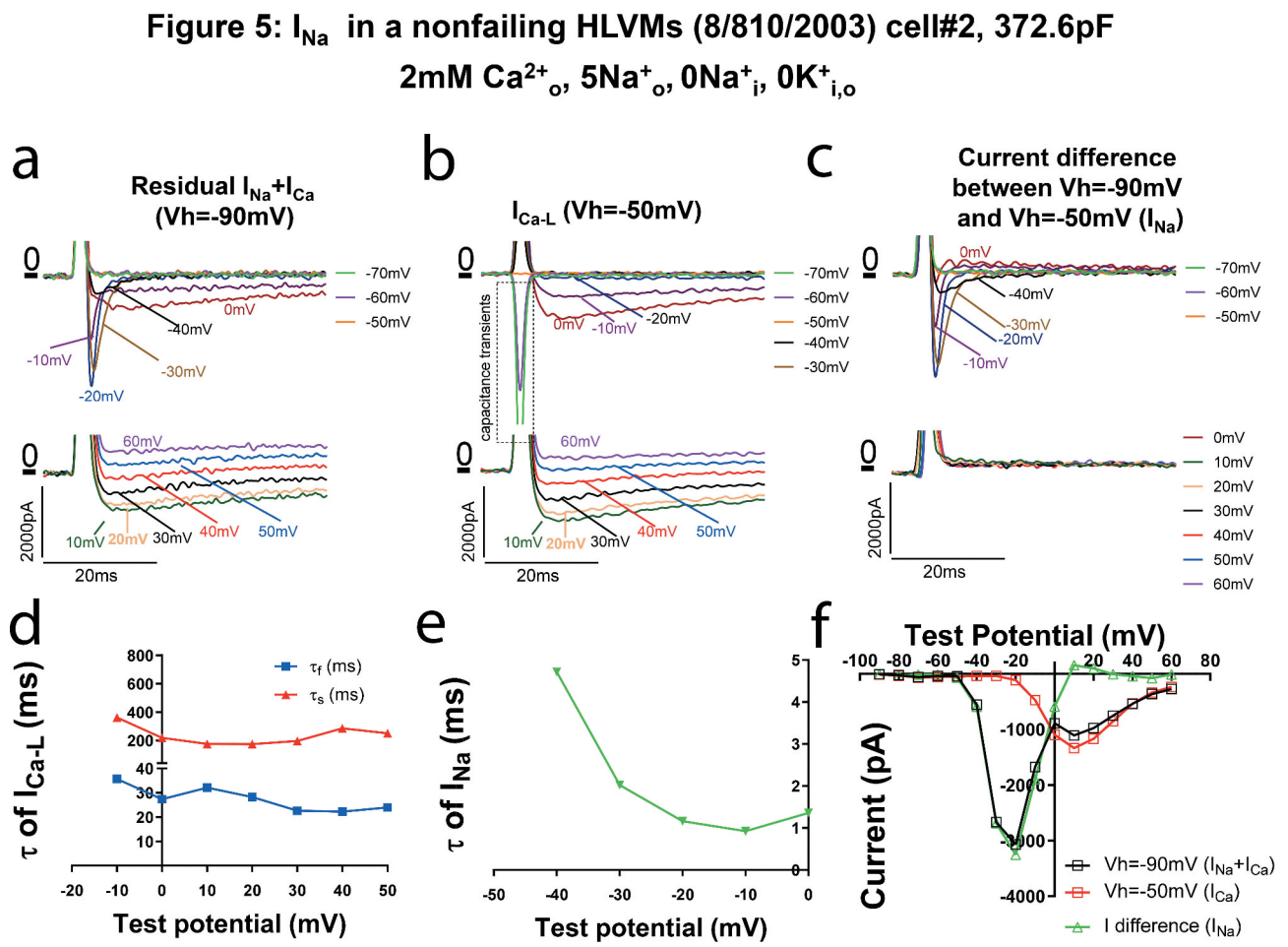


**Figure 3.** $I_{Ca-LVA}$ in a HLVM of a failing human heart due to primary pulmonary hypertension is insensitive to 100 μM TTX and inactivates faster than $I_{Ca-L}$. The HLVM was incubated in $Ca^{2+}$ current recording perfusate containing 100 μM TTX. **A-C**. Current traces at different test voltages (in a 10 mV-increment from the holding potential) of total $I_{Ca}$ recorded from the holding potential of −90 mV (a), of $I_{Ca-L}$ from the holding potential of −50 mV (b), of differential currents between currents recorded with the holding potential of −90 mV and currents recorded with the holding potential of −50 mV (c). In B, please note that the fast downward currents recorded were capacitance transients (currents charging and discharging the membrane) when we hyperpolarized the cell from −50 mV to −90 mV~-60 mV. (d). Fast and slow time constants ($\tau_f$ and $\tau_s$) of $I_{Ca-L}$ at different test potentials. (e). Time constants (τ) of $I_{Ca-LVA}$ at different test potentials. (f). The current (i)-Voltage (v) relationships of total $I_{Ca}$, $I_{Ca}$ recorded with the holding potential of −50 mV ($I_{Ca-L}$), differential currents between currents recorded with the holding potential of −90 mV and currents recorded with the holding potential of −50 mV ($I_{Ca-LVA}$).

We also tried to record $I_{Ca-LVA}$ current with a high extracellular $Ca^{2+}$ (10 mM) bath solution containing 0 $Na^+$ and 0 $K^+$, and 100 μM TTX in FHVMs and found one FHVM with $I_{Ca-LVA}$. The high extracellular $Ca^{2+}$ shifted the activation of the L-type and $I_{Ca-LVA}$ currents to more positive voltages due to the surface charge screening effect [34] (Figure 4). The decay of $I_{Ca-L}$ and $I_{Ca-LVA}$ was fit with a double- or single- exponential decay function, as shown in Figure 4d and e. It seemed that the τ values here were smaller than those in Figures 1 and 6, probably due to high extracellular $Ca^{2+}$ (10 mM). The peak $I_{Ca-LVA}$ was −4.58 pA/pF (Figure 4f), greater than the amplitudes of $I_{Ca-LVA}$ in the other two FHVMs recorded with bath solution containing 2 mM $Ca^{2+}$.

### *$I_{Ca-LVA}$ current in failing HVMs inactivates slower than $I_{Na}$*

We further compared the properties of $I_{Ca-LVA}$ current in the human ventricular myocytes with $I_{Na}$ in a nonfailing HLVMs recorded with low concentration of $Na^+$ (Figure 5). In current traces recorded at

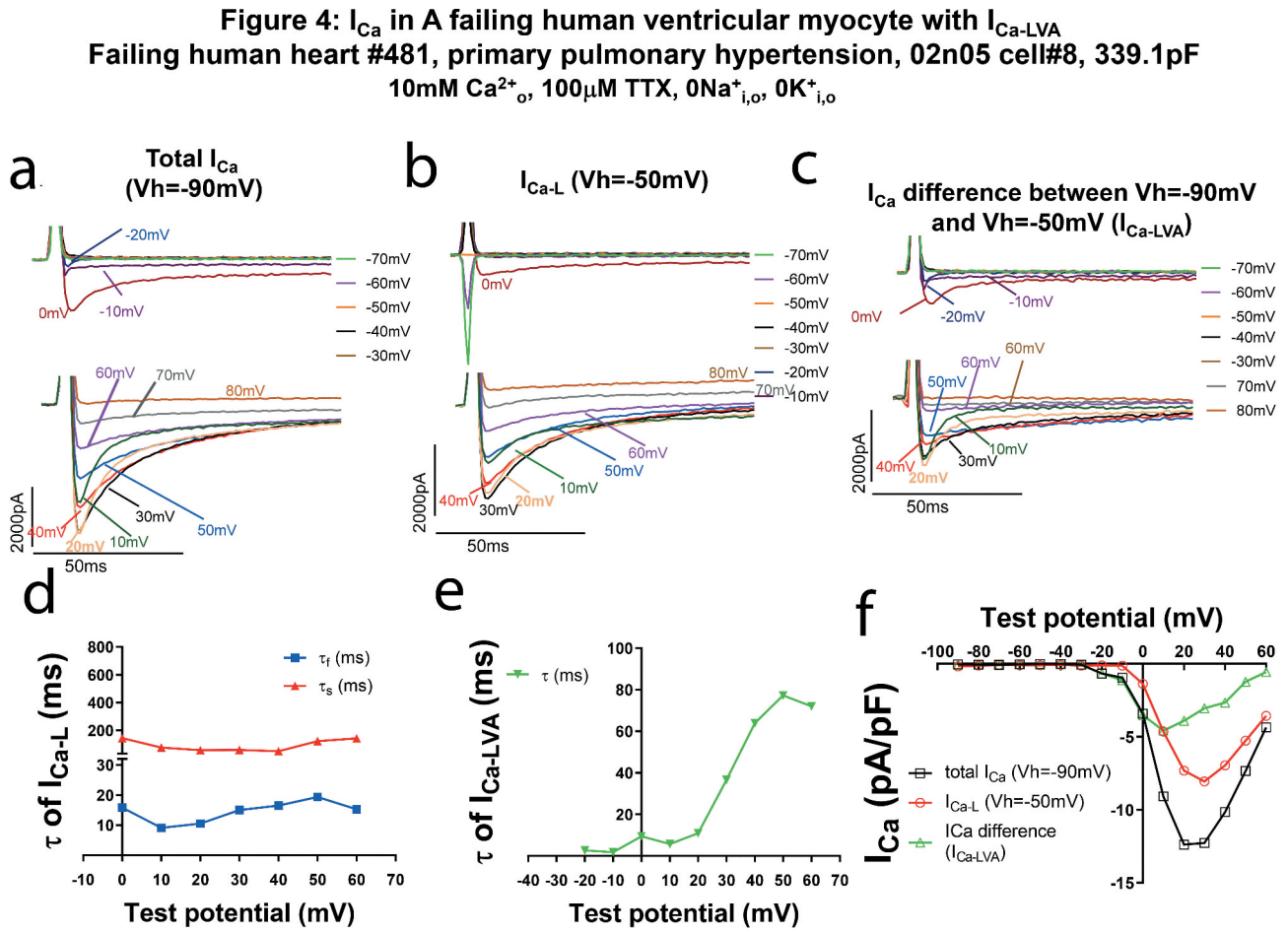


**Figure 4.** $I_{Ca-LVA}$ in a HLVM of a failing human heart due to primary pulmonary hypertension recorded with 10 mM extracellular $Ca^{2+}$ is insensitive to 100 μM TTX. A FHLVM was incubated in $Ca^{2+}$ current recording perfusate containing 10 mM $Ca^{2+}$ and 100 μM TTX. (a–c). Current traces at different test voltages (in 10 mV increment from the holding potential) of total $I_{Ca}$ recorded from the holding potential of −90 mV (a), of $I_{Ca}$ recorded with the holding potential of −50 mV (b, presumably $I_{Ca-L}$), and of differential currents between total $I_{Ca}$ and $I_{Ca-L}$ (c). In B, please note that the fast downward currents recorded were capacitance transients (currents charging and discharging the membrane) when we hyperpolarized the cell from −50 mV to −90 mV~-60 mV. (d). Fast and slow time constants ($\tau_f$ and $\tau_s$) of $I_{Ca-L}$ at different test potentials. (e). Time constants (τ) of $I_{Ca-LVA}$ at different test potentials. (f). The current (i)-Voltage (v) relationships of total $I_{Ca}$, $I_{Ca}$ recorded with the holding potential of −50 mV ($I_{Ca-L}$), and differential currents between currents recorded with the holding potential of −90 mV and currents recorded with the holding potential of −50 mV ($I_{Ca-LVA}$). The shift of I–V curves to more positive directions was due to high extracellular $Ca^{2+}$ causing the surface charge screening effect.

Vh = −90 mV ($I_{Ca}$+$I_{Na}$), there were large and quickly inactivated currents (Figure 5a), which was eliminated by the $V_h$ = −50 mV (Figure 5b, note that the downward currents in Figure 5b were the capacitance transient currents when hyperpolarizing the cell from −50 mV. They were neither $I_{Ca-LVA}$ nor $I_{Na}$). $I_{Na}$ was obtained by subtracting $I_{Ca}$ from total current and its inactivation was very fast (Figure 5c and f). $I_{Na}$ completely returned to baseline within 10 ms and the time constants were <5 ms at all test potentials (Figure 5c and e), which were an order smaller than the time constants of $I_{Ca-LVA}$ (Figure 5e versus Figure 3e) and the fast time constants of $I_{Ca-L}$ (Figure 5d). These results suggest that the $I_{Ca-LVA}$ currents observed in failing human ventricular myocytes are not $I_{Na}$.

### *$I_{Ca-LVA}$ current in comparison with $I_{Ca-T}$ mediated by Cav3.2/α1 H in mouse ventricular myocytes*

We intended to compare properties of $I_{Ca-LVA}$ recorded in HLVMs and those of $I_{Ca-T}$ in transgenic mouse ventricular myocytes expressing human Cav3.2/α1 H. In VMs isolated from these transgenic mice, $I_{Ca-T}$ can be separated by holding the cell at −90 mV versus at −50 mV (Figure 6a–c, f). The inactivation time constants (Figure 6e) and voltage

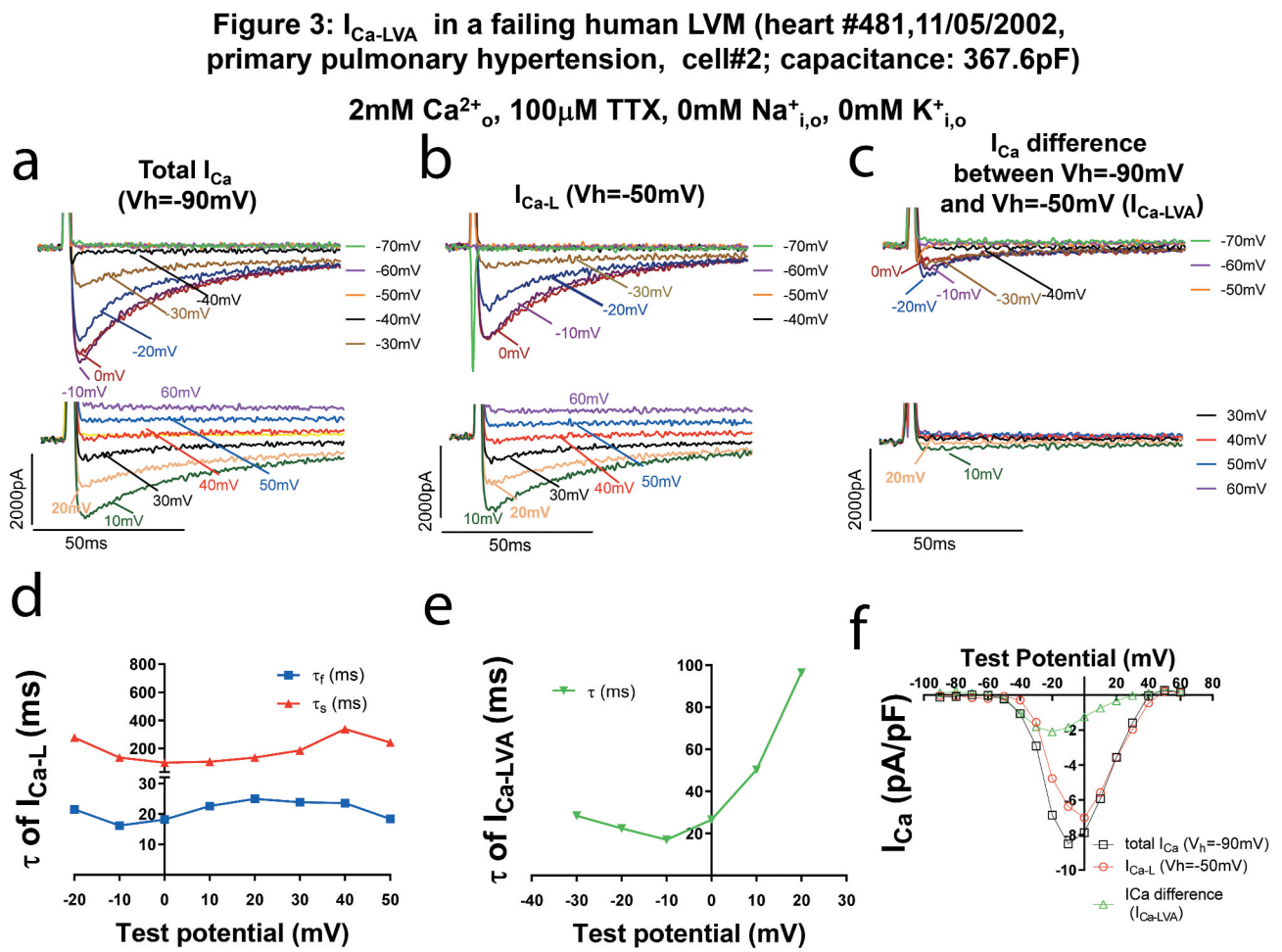


**Figure 5.** Sodium current ($I_{Na}$) in a nonfailing HLVMs recorded with 5 mM extracellular $Na^+$ added to $Ca^{2+}$ current recording solution. (a–c). Current traces recorded at different test voltages (in 10 mV increment from the holding potential) of total $I_{Ca}$ with $I_{Na}$ current contamination recorded with the holding potential of −90 mV (a), $I_{Ca}$ recorded with the holding potential of −50 mV when sodium channels were inactivated (b, presumably $I_{Ca-L}$), differential currents between currents recorded with the holding potential of −90 mV and currents recorded with the holding potential of −50 mV (presumably $I_{Na}$, (c). In B, please note that the fast downward currents recorded were capacitance transients (currents charging and discharging the membrane) when we hyperpolarized the cell from −50 mV to −90 mV~-60 mV. (d). Fast and slow time constants ($\tau_f$ and $\tau_s$) of $I_{Ca-L}$ at different test potentials. (e). Time constants (τ) of $I_{Na}$ at different test potentials. $I_{Na}$ could be fit well with a single exponential decay function. τ values were much smaller than $\tau_f$ of $I_{Ca-L}$ or $I_{Ca-T}$. (f). Current (i)-Voltage (v) relationships of total currents containing both $I_{Ca}$ and $I_{Na}$, $I_{Ca}$ recorded with the holding potential of −50 mV ($I_{Ca-L}$), and differential currents between currents recorded with the holding potential of −90 mV and currents recorded with the holding potential of −50 mV (presumably $I_{Na}$).

dependency (figure 6f) of $I_{Ca-T}$ mediated by human Cav3.2/α1 H in mouse VMs were like $I_{Ca-LVA}$ current observed in human VMs. However, there was an extra sustained component in $I_{Ca-LVA}$ in HLVMs in Figures 1, 3 and 4, which could be mediated by Cav1.3.

## Discussion

### *Few human LVMs have $I_{Ca-LVA}$ current*

The existence of $I_{Ca-T}$ current in human ventricular myocytes were explored in myocytes isolated from failing or nonfailing human hearts and no $I_{Ca-T}$ was found previously [14]. No Cav1.3 $I_{Ca-LVA}$ was found in human ventricular myocytes previously [35] but Cav1.3 protein was shown in human ventricular tissue [23]. In this study, we examined substantial number of human left ventricular myocytes from nonfailing hearts, failing hearts and failing hearts with LVAD support to find $I_{Ca-LVA}$ with whole-cell voltage-clamp technique. We found $I_{Ca-LVA}$ insensitive to 100 μM TTX, having the activation threshold at ~-50 mV, peaked around −30 mV and inactivating at rates faster than Cav1.2 $I_{Ca-L}$ but slower than $I_{Na}$, in 3

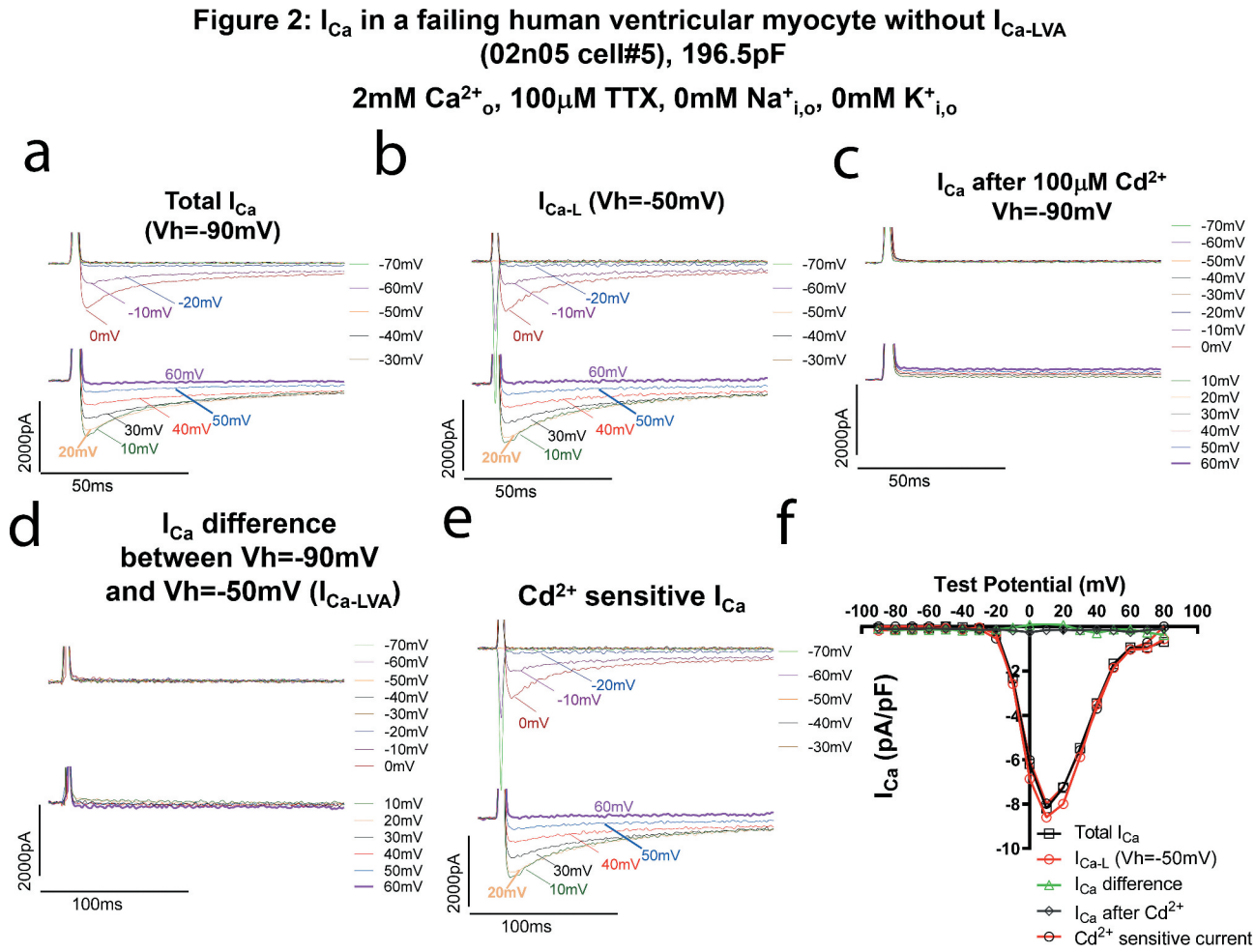


**Figure 6.** $I_{Ca-T}$ mediated by human T-type $Ca^{2+}$ channel Cav3.2/α1 H in transgenic mouse ventricular myocytes cardiac-specifically overexpressing α1 H. (a–c). Current traces at different test voltages (in 10 mV increment from the holding potential) of total $I_{Ca}$ recorded from the holding potential of −90 mV (a), of $I_{Ca}$ recorded with the holding potential of −50 mV when $I_{Ca-T}$ was inactivated (b, presumably $I_{Ca-L}$), of differential currents between currents recorded with the holding potential of −90 mV and currents recorded with the holding potential of −50 mV (presumably $I_{Ca-T}$, (c). In B, please note that the fast downward currents recorded were capacitance transients (currents charging and discharging the membrane) when we hyperpolarized the cell from −50 mV to −90 mV~-60 mV. (d). Fast and slow time constants ($\tau_f$ and $\tau_s$) of $I_{Ca-L}$ at different test potentials. (e). Time constants (τ) of $I_{Ca-T}$ at different test potentials. $I_{Ca-T}$ could be fit well with single exponential decay. (f). The current (i)-voltage (v) relationships of total currents containing both $I_{Ca}$ and $I_{Na}$, $I_{Ca}$ recorded with the holding potential of −50 mV ($I_{Ca-L}$), differential currents between currents recorded with the holding potential of −90 mV and currents recorded with the holding potential of −50 mV (presumably $I_{Na}$).

LVMs from 2 failing human hearts but not from nonfailing and failing with LVAD-support human hearts. These properties were different from Cav1.2 L-type $Ca^{2+}$ currents or $Na^+$ currents and more consistent properties of $I_{Ca-LVA}$ current (Cav1.3 or Cav3 or both). However, the existence of $I_{Ca-LVA}$ was found in only few HLVMs of few human hearts. $I_{Ca-LVA}$ in 1 HLVM was detected in the presence of 100 μM $CdCl_2$, suggesting that in that cell $I_{Ca-LVA}$ was more likely $I_{Ca-T}$, as Cav1.3 mediated $I_{Ca}$ can be well blocked by 100 μM $CdCl_2$ [25]. However, in the other two cells with $I_{Ca-LVA}$, we did not have the chance to determine $I_{Ca-LVA}$ sensitivity to $Cd^{2+}$ or $Ni^{2+}$. Thus we could not determine which subtype of LVA $Ca^{2+}$ channels (Cav1.3 or Cav3.1 or Cav3.2 or Cav3.3) [36] was expressed in these myocytes.

### *What kind of human LVMs were expressing $I_{Ca-LVA}$?*

Firstly, it is clear that $I_{Ca-LVA}$ was only found in nonischemic failing HLVMs. However, statistical analysis did not identify a significant correlation between the expression of $I_{Ca-LVA}$ and the disease states (F, NF or F-LVAD) or between $I_{Ca-LVA}$ and

etiologies (ischemic or nonischemic). Our data suggest that the existence of $I_{Ca\text{-}LVA}$ in adult human LVMs is rare and happened to be in non-ischemic failing human LVMs.

Secondly, these HLVMs with $I_{Ca\text{-}LVA}$ were probably not myocytes with automaticity. Within the ventricles, $I_{Ca\text{-}LVA}$ current could be present in Purkinje cells. However, according to our isolation procedure, human LVMs used in this study were from the middle wall where Purkinje cells were absent [37]. In addition, these myocytes might not be Purkinje cells because they had a rod shape, dense myofibrils and clear striations while human Purkinje cells are long, narrow spindle-shaped and with sparse myofibrils [38]. However, we could not rule out that these myocytes could be transition myocytes between Purkinje myocytes and ventricular myocytes.

### What are the roles of $I_{Ca\text{-}LVA}$ in failing human LVMs?

In fetal hearts, TTCCs clearly participate in cardiac excitation-contraction coupling [6]. We showed that in adult mouse myocytes, TTCCs do not play a significant role in inducing excitation-contraction coupling process. Nor does $Ca^{2+}$ influx through TTCCs effectively load the sarcoplasmic reticulum [16]. In normal adult ventricular myocytes, TTCC expression is mostly absent except for few species [12,13].

TTCC expression reappears in stressed myocardium [2,19]. T-type $Ca^{2+}$ current has been reported to be increased in genetically hypertrophied hamster hearts [39]. Multiple hormones (e.g., angiotensin II, aldosterone), growth factors (e.g., IGF-1) and transcription factors (e.g., NRSF and Nkx2.5) involved in cardiac disease progression have been shown to regulate the expression of TTCCs in cardiomyocytes [2]. However, the pathological significance of TTCC reappearance in diseased hearts is not clear. It may modulate the excitation-contraction process by enhancing the amplification of $Ca^{2+}$-induced $Ca^{2+}$ release and modestly increasing SR $Ca^{2+}$ content [15]. The loss of Cav3.1 in mouse hearts exacerbated cardiac function after myocardial infarction [40]. Cav3.2 is necessary for cardiac hypertrophy [2] but the increase of $Ca^{2+}$ influx through Cav3.1 antagonizes cardiomyocyte hypertrophy [21] rather than promotes cardiomyocyte hypertrophy as increased $Ca^{2+}$ influx through the LTCC does [41]. Additionally, TTCC reappearance may contribute to cardiac arrhythmias observed in stressed hearts because the T- type $Ca^{2+}$ channel blockers reduced arrhythmias independent of their L-type $Ca^{2+}$channel blocker activity [42]. Another possibility is that TTCC re-expression is simply part of re-expression of the fetal gene program in diseased hearts. In addition, the expression of TTCCs is associated with cell cycle [2] and we could not rule out these human LVMs with $I_{Ca\text{-}LVA}$ were also associated with cell cycle activity.

If the $I_{Ca\text{-}LVA}$ observed in our study was Cav1.3 mediated, its significance needs to be further determined. It has been reported that in atrial myocytes Cav1.3 couples with small conductance $Ca^{2+}$-activated $K^+$ channels and the loss of Cav1.3 prolongs the action potential [23]. However, the role of Cav1.3 in the ventricles has not been well studied and the physiological and pathological relevance of Cav1.3 in HLVMs warrants further study.

Though we only found 1 out of 7 LVMs from a failing heart with idiopathic cardiomyopathy and another 2 out of 8 LVMs from another failing heart with primary pulmonary hypertension had $I_{Ca\text{-}LVA}$, the absolute number of LVMs expressing $I_{Ca\text{-}LVA}$ in these two specific hearts could be large as the total myocyte number in human hearts is huge. It is highly likely that TTCCs in these two hearts play some physiological and/or pathological roles.

### Limitations

Our study indicates that a very minimal but still detectable amounts of human myocytes had $I_{Ca\text{-}LVA}$. While all 3 LVMs with $I_{Ca\text{-}LVA}$ were from failing human hearts, statistically we did not detect difference in possibilities of $I_{Ca\text{-}LVA}$ expression among nonfailing, failing and failing with LVAD support hearts. On the other hand, as the detection method used was low throughput and human left ventricle has a huge number of cardiomyocytes, the hearts deemed without $I_{Ca\text{-}LVA}$ in LVMs may actually contain few myocytes with $I_{Ca\text{-}LVA,}$ which could be easily missed by our low throughput detection. It is also possible that the expression/

re-expression of $I_{Ca\text{-}LVA}$ in VMs were restricted in a specific area of the ventricles and the midwall of the free wall of the LV surveyed by us had less percentage of myocytes expressing $I_{Ca\text{-}LVA}$. Furthermore, there was rundown of $I_{Ca\text{-}T}$ [32] and the amplitude of $I_{Ca\text{-}LVA}$ could be too small to be confidently measured in some LVMs. Taken together, this study may underestimate the percentage of hearts with $I_{Ca\text{-}LVA}$ and the re-expression of $I_{Ca\text{-}LVA}$ in some failing human hearts may play important pathological roles. At last, this study was to retrospectively examine the existence of $I_{Ca\text{-}LVA}$ and key experiments for determining the nature of the current, such as the application of low concentrations of $Ni^{2+}$, $Cd^{2+}$ and dihydropyridine blockers, were not performed. The biophysical properties, including voltage dependent activation and inactivation, and time dependent decay, of Cav1.3 and Cav3 $Ca^{2+}$ channels overlap [2,22]. Therefore, we could not determine if these currents were mediated by Cav1.3 or Cav3 or both.

## *Conclusion*

We recorded a low voltage-activated $Ca^{2+}$ currents in few human ventricular myocytes of few human hearts that happened to be nonischemic failing.

## Disclosure statement

No potential conflict of interest was reported by the authors.

## Funding

This research was partially supported by grants from the National Institutes of Health (HL088243 and HL140071 to X.C. and HL139960 to S.R.H).

## ORCID

Xiaoying Zhang http://orcid.org/0000-0002-8197-6798

## References

[1] Bers DM. Calcium cycling and signaling in cardiac myocytes. Annu Rev Physiol. 2008;70:23–49.
[2] Ono K, Iijima T. Cardiac T-type Ca(2+) channels in the heart. J Mol Cell Cardiol. 2010;48:65–70.
[3] Vassort G, Talavera K, Alvarez JL. Role of T-type Ca2+ channels in the heart. Cell Calcium. 2006;40: 205–220
[4] Mangoni ME, Couette B, Bourinet E, et al. Functional role of L-type Cav1.3 Ca2+ channels in cardiac pacemaker activity. Proc Natl Acad Sci U S A. 2003;100:5543–5548.
[5] Yasui K, Niwa N, Takemura H, et al. Pathophysiological significance of T-type Ca2+ channels: expression of T-type Ca2+ channels in fetal and diseased heart. J Pharmacol Sci. 2005;99:205–210.
[6] Tohse N, Seki S, Kobayashi T, et al. Development of excitation-contraction coupling in cardiomyocytes. Jpn J Physiol. 2004;54:1–6.
[7] Mizuta E, Shirai M, Arakawa K, et al. Different distribution of Cav3.2 and Cav3.1 transcripts encoding T-type Ca(2+) channels in the embryonic heart of mice. Biomed Res. 2010;31:301–305.
[8] Qu Y, Boutjdir M. Gene expression of SERCA2a and L- and T-type Ca channels during human heart development. Pediatr Res. 2001;50:569–574.
[9] Li Y, Zhang X, Zhang C, et al. Increasing T-type calcium channel activity by beta-adrenergic stimulation contributes to beta-adrenergic regulation of heart rates. J Physiol. 2018;596:1137–1151.
[10] Xu X, Best PM. Postnatal changes in T-type calcium current density in rat atrial myocytes. J Physiol. 1992;454:657–672.
[11] Leuranguer V, Monteil A, Bourinet E, et al. T-type calcium currents in rat cardiomyocytes during postnatal development: contribution to hormone secretion. Am J Physiol Heart Circ Physiol. 2000;279:H2540–8.
[12] Droogmans G, Nilius B. Kinetic properties of the cardiac T-type calcium channel in the guinea-pig. J Physiol. 1989;419:627–650.
[13] Tytgat J, Nilius B, Vereecke J, et al. The T-type Ca channel in guinea-pig ventricular myocytes is insensitive to isoproterenol. Pflugers Arch. 1988; 411:704–706.
[14] Beuckelmann DJ, Nabauer M, Erdmann E. Characteristics of calcium-current in isolated human ventricular myocytes from patients with terminal heart failure. J Mol Cell Cardiol. 1991;23:929–937.
[15] Sipido KR, Carmeliet E, Van de Werf F. T-type Ca2+ current as a trigger for Ca2+ release from the sarcoplasmic reticulum in guinea-pig ventricular myocytes. J Physiol. 1998;508;(Pt 2):439–451. DOI:10.1111/j.1469-7793.1998.439bq.x
[16] Jaleel N, Nakayama H, Chen X, et al. Ca2+ influx through T- and L-type Ca2+ channels have different effects on myocyte contractility and induce unique cardiac phenotypes. Circ Res. 2008;103:1109–1119.
[17] Guo W, Kamiya K, Kodama I, et al. Cell cycle-related changes in the voltage-gated Ca2+ currents in cultured newborn rat ventricular myocytes. J Mol Cell Cardiol. 1998;30:1095–1103.
[18] Chen X, Wilson RM, Kubo H, et al. Adolescent feline heart contains a population of small, proliferative ventricular myocytes with immature physiological properties. Circ Res. 2007;100:536–544.

[19] Rubi L, Todt H, Kubista H, et al. Calcium current properties in dystrophin-deficient ventricular cardiomyocytes from aged mdx mice. Physiol Rep. 2018;6 (1):e13567.
[20] Chiang CS, Huang CH, Chieng H, et al. The Ca(v)3.2 Ca V 3.2 T-Type Ca(2+) Ca + channel is required for pressure overload-induced cardiac hypertrophy in mice. Circ Res. 2009;104(4):522–530. .
[21] Nakayama H, Bodi I, Correll RN, et al. alpha1G-dependent T-type Ca2+ current antagonizes cardiac hypertrophy through a NOS3-dependent mechanism in mice. J Clin Invest. 2009;119:3787–3796.
[22] Zamponi GW, Striessnig J, Koschak A, et al. The physiology, pathology, and pharmacology of voltage-gated calcium channels and their future therapeutic potential. Pharmacol Rev. 2015;67:821–870.
[23] Lu L, Zhang Q, Timofeyev V, et al. Molecular coupling of a Ca2+-activated K+ channel to L-type Ca2+ channels via alpha-actinin2. Circ Res. 2007;100:112–120.
[24] Lipscombe D, Helton TD, Xu W. L-type calcium channels: the low down. J Neurophysiol. 2004;92:2633–2641.
[25] Vivas O, Moreno CM, Santana LF, et al. Proximal clustering between BK and CaV1.3 channels promotes functional coupling and BK channel activation at low voltage. Elife. 2017;6.
[26] Pidoplichko VI. Two different tetrodotoxin-separable inward sodium currents in the membrane of isolated cardiomyocytes. Gen Physiol Biophys. 1986;5:593–604.
[27] Lemaire S, Piot C, Seguin J, et al. Tetrodotoxin-sensitive Ca2+ and Ba2+ currents in human atrial cells. Recept Channels. 1995;3:71–81.
[28] Piacentino V 3rd, Gaughan JP, Houser SR. L-type Ca(2 +) currents overlapping threshold Na(+) currents: could they be responsible for the “slip-mode” phenomenon in cardiac myocytes? Circ Res. 2002;90:435–442.
[29] Chen X, Piacentino V 3rd, Furukawa S, et al. L-type Ca2+ channel density and regulation are altered in failing human ventricular myocytes and recover after support with mechanical assist devices. Circ Res. 2002;91:517–524.
[30] Sanbe A, Gulick J, Hanks MC, et al. Reengineering inducible cardiac-specific transgenesis with an attenuated myosin heavy chain promoter. Circ Res. 2003;92:609–616.
[31] Tang M, Zhang X, Li Y, et al. Enhanced basal contractility but reduced excitation-contraction coupling efficiency and beta-adrenergic reserve of hearts with increased Cav1.2 activity. Am J Physiol Heart Circ Physiol. 2010;299:H519–28.
[32] Li Y, Wang F, Zhang X, et al. beta-adrenergic stimulation increases Cav3.1 activity in cardiac myocytes through protein kinase A. PLoS One. 2012;7:e39965.
[33] Hering J, Feltz A, Lambert RC. Slow inactivation of the Ca(V)3.1 isotype of T-type calcium channels. J Physiol. 2004;555:331–344.
[34] Fukushima Y, Hagiwara S. Currents carried by monovalent cations through calcium channels in mouse neoplastic B lymphocytes. J Physiol. 1985; 358:255–284.
[35] Mangoni ME, Couette B, Marger L, et al. Voltage-dependent calcium channels and cardiac pacemaker activity: from ionic currents to genes. Prog Biophys Mol Biol. 2006;90:38–63.
[36] Lee JH, Gomora JC, Cribbs LL, et al. Nickel block of three cloned T-type calcium channels: low concentrations selectively block alpha1H. Biophys J. 1999;77:3034–3042.
[37] Ohkawa S. Distribution of Purkinje cells in hearts of human and various animals. J Arrhythm. 2008;24:177–179.
[38] Han W, Zhang L, Schram G, et al. Properties of potassium currents in Purkinje cells of failing human hearts. Am J Physiol Heart Circ Physiol. 2002;283:H2495–503.
[39] Sen L, Smith TW. T-type Ca2+ channels are abnormal in genetically determined cardiomyopathic hamster hearts. Circ Res. 1994;75:149–155.
[40] Le Quang K, Naud P, Qi XY, et al. Role of T-type calcium channel subunits in post-myocardial infarction remodelling probed with genetically engineered mice. Cardiovasc Res. 2011;91:420–428.
[41] Chen X, Nakayama H, Zhang X, et al. Calcium influx through Cav1.2 is a proximal signal for pathological cardiomyocyte hypertrophy. J Mol Cell Cardiol. 2011;50:460–470.
[42] Kinoshita H, Kuwahara K, Takano M, et al. T-type Ca2 + channel blockade prevents sudden death in mice with heart failure. Circulation. 2009;120:743–752.