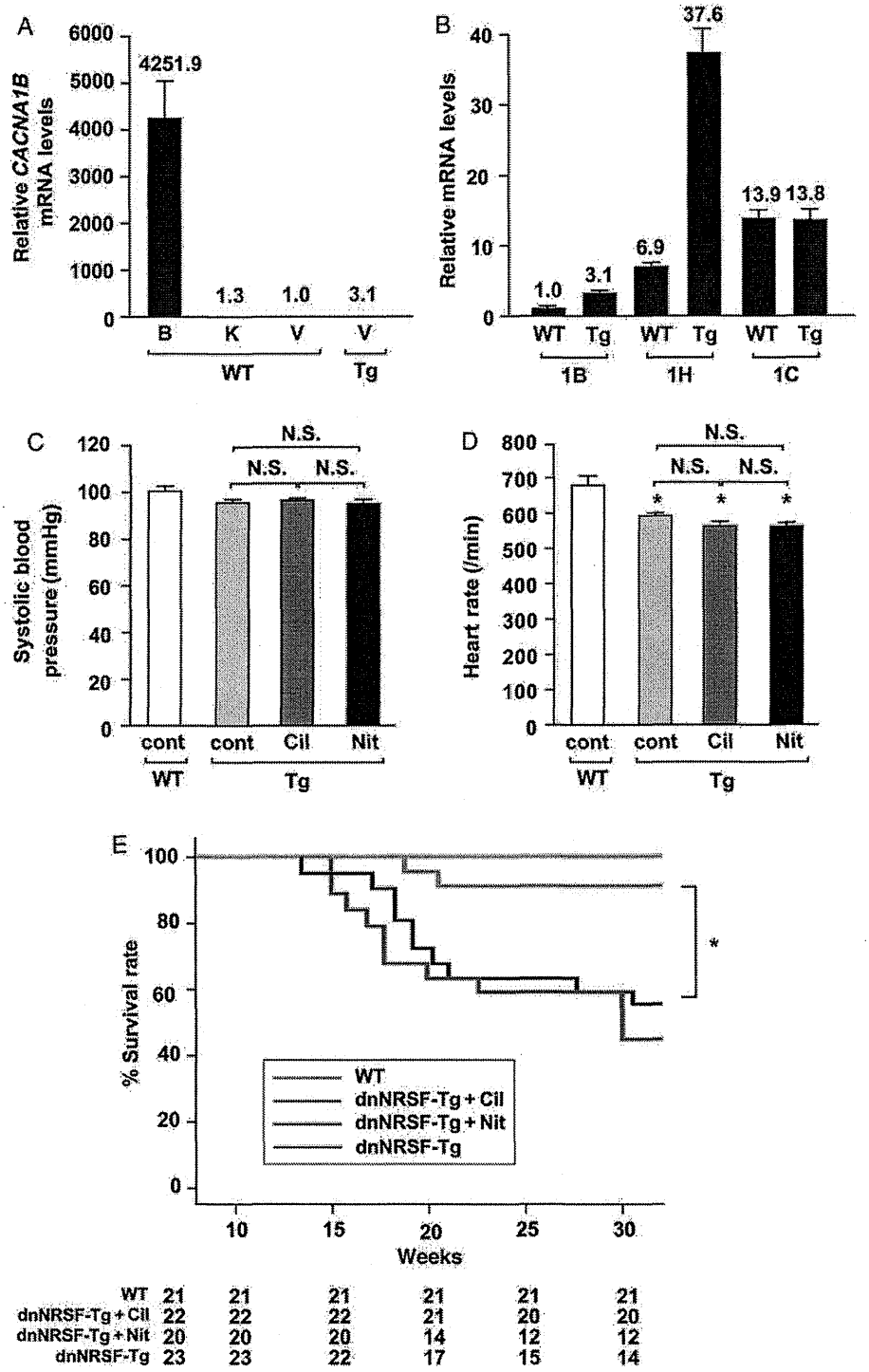

Figure 1 Pharmacological blockade of NCCs by cilnidipine improves survival among dnNRSF-Tg mice. (*A*) Relative levels of CACNA1B mRNA in brains (B) from WT, kidney (K) from WT, cardiac ventricle (V) from WT, and cardiac ventricle (V) from 8-week-old dnNRSF-Tg mice (Tg); levels in cardiac ventricle from WT mice were assigned a value of 1.0. $n = 3$ each for brain, kidney, and cardiac ventricle from WT mice and $n = 2$ for cardiac ventricle from dnNRSF-Tg mice. (*B*) Relative levels of CACNA1B, CACNA1H, and CACNA1C mRNA in cardiac ventricle from 8-week-old WT mice and dnNRSF-Tg mice (Tg); levels of CACNA1B mRNA in WT mice were assigned a value of 1.0. $n = 5$ for WT mice and $n = 7$ for dnNRSF-Tg. (*C* and *D*) Systolic blood pressures (*C*) and heart rates (*D*) in 20-week-old untreated WT, untreated Tg (Tg-cont), cilnidipine-treated Tg (Tg-Cil), and nitrendipine-treated Tg-Nit mice ($n = 15$ each for untreated Tg, Tg-Cil, and Tg-Nit, and $n = 10$ for untreated WT). ANOVA with post hoc Fisher's tests was used for analysis. $^*P < 0.05$. N.S.: not significant. (*E*) Kaplan–Meyer survival curves for untreated WT, untreated Tg, Cil-treated Tg, and Nit-treated Tg over a 24-week drug administration period (from 8 to 32 weeks of age): Log-rank test was used for analysis. $^*P < 0.05$ ($n = 21$ for WT, $n = 23$ for Tg without drugs, $n = 22$ for Tg + Cil, and $n = 20$ for Tg + Nit). The numbers of mice alive in each group at the end of each period are shown at the bottom of the figure. All data except survival curves are shown as means ± SEM.

of dnNRSF-Tg mice, though blood pressures were slightly lower and heart rates were significantly slower in dnNRSF-Tg mice than in untreated WT mice, as previously reported (systolic blood pressure: WT, 101.40 ± 1.48; Tg, 96.0 ± 1.75; Tg + cilnidipine, 96.67 ± 1.64; Tg + nitrendipine, 95.47 ± 1.92 mmHg and Heart rates: WT, 682.3 ± 27; dnNRSF-Tg, 590.6 ± 10.9; Tg + cilnidipine, 567.13 ± 17.58; Tg + nitrendipine, 568.8 ± 11.07/min) (*Figure 1C* and *D*).[8] We found that cilnidipine dramatically improved the survival rate among dnNRSF-Tg mice, compared with mice treated with nitrendipine or untreated control (*Figure 1E*). Although heart-to-body weight ratios were higher in dnNRSF-Tg than in WT mice, as reported previously,[8] heart-to-body weight ratios did not significantly differ among the control, cilnidipine, and nitrendipine groups of dnNRSF-Tg mice (WT, 4.08 ± 0.31; Tg, 5.94 ± 0.24; Tg + cilnidipine, 5.61 ± 0.48; Tg + nitrendipine, 5.94 ± 0.36 mg/g) (*Figure 2A*). Lung-to-body weight ratios also did not differ among these three groups (WT, 5.28 ± 0.37; Tg, 6.07 ± 0.22; Tg + cilnidipine, 5.93 ± 0.79; Tg + nitrendipine, 5.9 ± 0.29 mg/g) (*Figure 2B*). In addition, histological analyses, including determination of the %fibrotic area, and echocardiographic analyses also showed no significant differences among these three groups (*Figure 2C–F* and *Table 1*). In contrast, the echocardiography and histology showed that, compared with untreated WT mice, left ventricular systolic function was diminished and %fibrotic area was increased in dnNRSF-Tg mice, as reported previously (*Figure 2C–F* and *Table 1*).[8] Consistent with these findings, there was no significant difference in the expression of two cardiac stress marker genes, *ANP* and *SERCA2*, among the three groups, whereas their expression did differ between untreated WT mice and dnNRSF-Tg mice, as described previously (*Figure 2G* and *H*).[8]

Expression of the fibrosis-related genes *Col1a1*, *Col3a1*, and *FN1*, encoding collagen type1α1, collagen type3α1, and fibronectin 1, respectively, was not affected by the drug treatments (see Supplementary material online, *Figure S1A–C*). Expression of genes encoding the fetal-type ion channels *CACNA1H*, *HCN2*, and *HCN4* was higher in untreated dnNRSF-Tg ventricles than in control WT ventricles, as reported previously, and cilnidipine did not affect expression of these genes in dnNRSF-Tg ventricles (see Supplementary material online, *Figure S1D–F*). Collectively, all of these data indicate that cilnidipine suppresses sudden death in dnNRSF-Tg mice without significantly affecting cardiac structure or function.

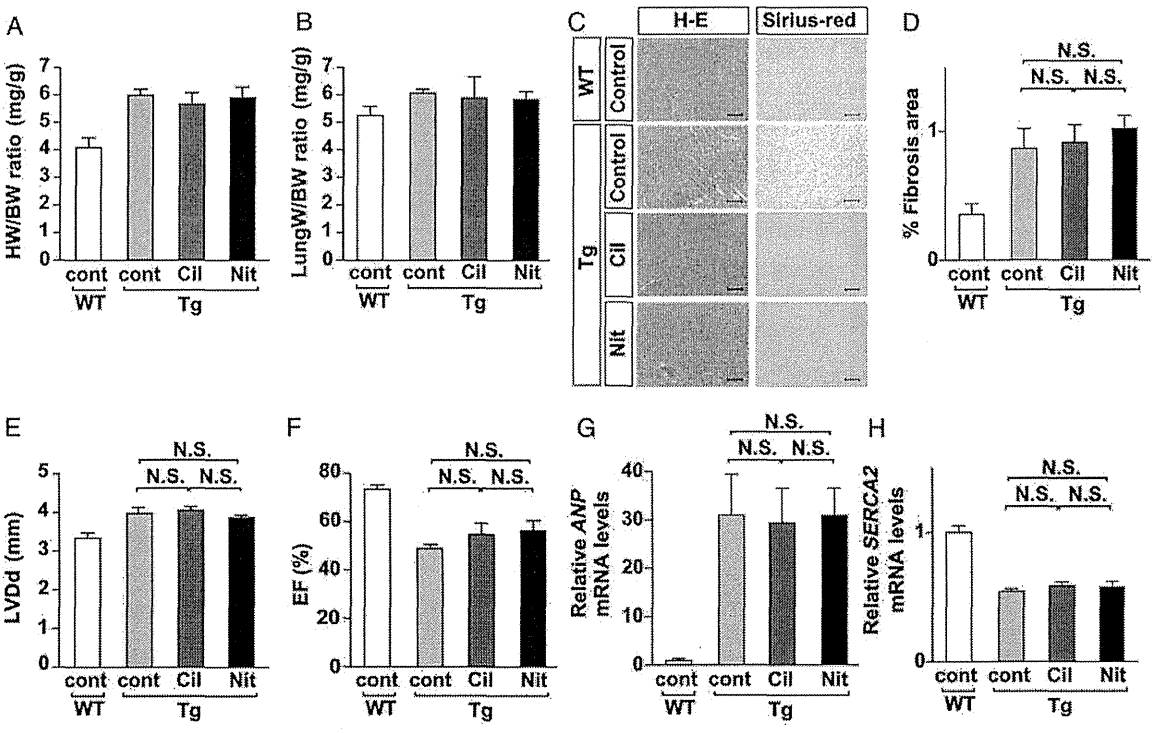

Figure 2 Cilnidipine does not affect cardiac structure or function in dnNRSF-Tg mice. (*A* and *B*) Heart-to-body weight (HW/BW) ratios (*A*) and lung-to-body weight (LungW/BW) ratios (*B*) in 20-week-old untreated WT (WT-cont), untreated Tg (Tg-cont), Cil-treated Tg (Tg-Cil), and Nit-treated Tg (Tg-Nit) mice ($n = 5$ for untreated WT, $n = 4$ for Tg-cont, $n = 4$ for Tg-Cil, and $n = 3$ for Tg-Nit). (*C*) Histology of hearts from 20-week-old untreated WT, Tg-cont, Tg-Cil, and Tg-Nit mice: H-E, haematoxylin-eosin staining; Sirius-red, Sirius-red staining. Scale bars = 100 μm. (*D*) %fibrotic area in 20-week-old untreated WT, Tg-cont, Tg-Cil, and Tg-Nit mice ($n = 5$ for Tg-cont, $n = 7$ for Tg-Cil). N.S.: not significant. (*E* and *F*) LVDd (*E*) and EF (*F*) assessed echocardiographically in untreated WT, Tg-cont, Tg-Cil, and Tg-Nit mice. $^*P < 0.05$. N.S.: not significant. ($n = 5$ each for untreated WT, Tg-cont, and Tg-Cil; $n = 7$ for Tg-Nit). (*G* and *H*) Relative levels of ANP (*G*) and SERCA2 (*H*) mRNA in cardiac ventricles from untreated WT, Tg-cont, Tg-Cil, and Tg-Nit mice; levels in untreated WT were assigned a value of 1.0. N.S.: not significant. ($n = 4$ each). ANOVA with post hoc Fisher's tests was used for analysis. All data are shown as means ± SEM.

Table 1 Echocardiographic parameters in 20-week-old mice

	WT	dnNRSF-Tg		
	Control	Cont	Cil	Nit
Pharmacological inhibition				
LVDd (mm)	3.3 ± 0.13	3.9 ± 0.19	4.0 ± 0.11	3.8 ± 0.08
LVDs (mm)	2.1 ± 0.08	3.1 ± 0.17	3.1 ± 0.11	2.9 ± 0.10
IVST (mm)	0.76 ± 0.02	0.72 ± 0.02	0.72 ± 0.02	0.71 ± 0.03
PWT (mm)	0.76 ± 0.02	0.74 ± 0.02	0.76 ± 0.02	0.76 ± 0.03
FS (%)	36.1 ± 2.3	20.3 ± 1.4	23.3 ± 2.7	23.8 ± 2.4
EF (%)	73.2 ± 2.7	49.0 ± 2.3	55.4 ± 4.2	57.0 ± 4.3
			dnNRSF-Tg	
	$1B^{+/+}$	$1B^{+/-}$	$1B^{+/+}$	$1B^{+/-}$
Genetic titration				
LVDd (mm)	3.2 ± 0.10	3.3 ± 0.08	4.1 ± 0.12	3.3 ± 0.07*
LVDs (mm)	2.2 ± 0.12	2.2 ± 0.06	3.2 ± 0.13	2.3 ± 0.08*
IVST (mm)	0.66 ± 0.01	0.68 ± 0.02	0.66 ± 0.02	0.69 ± 0.02
PWT (mm)	0.68 ± 0.02	0.67 ± 0.02	0.66 ± 0.02	0.68 ± 0.02
FS (%)	31.8 ± 1.8	33.1 ± 1.9	20.4 ± 1.3	30.4 ± 1.3*
EF (%)	66.4 ± 2.4	68.9 ± 2.6	49.0 ± 2.4	64.3 ± 1.8*

Values are means ± SEM. Cil, cilnidipine; Nit, nitrendipine; $1B^{+/+}$, $CACNA1B^{+/+}$; $1B^{+/-}$, $CACNA1B^{+/-}$; LVDd, left ventricular diastolic dimension; LVDs, left ventricular systolic dimension; FS, fractional shortening; IVST, intraventricular septum wall thickness; PWT, posterior wall thickness. Numbers of mice tested in the pharmacological inhibition study are as follows: $n = 5$ for WT, untreated dnNRSF-Tg, and Cil-treated dnNRSF-Tg; $n = 7$ for Nit-treated dnNRSF-Tg (upper panel). Numbers of mice tested in the genetic titration study are as follows: $n = 13$ for $1B^{+/+}$, $n = 14$ for $1B^{+/-}$, $n = 11$ for dnNRSF-Tg;$1B^{+/+}$, and $n = 15$ for dnNRSF-Tg; $1B^{+/-}$ (lower panel). ANOVA with *post hoc* Fisher's tests was used for the analysis.
*$P < 0.05$ vs. dnNRSF-Tg;$1B^{+/+}$.

3.2 Cilnidipine improves cardiac autonomic nervous system function and reduces arrhythmicity in dnNRSF-Tg mice

We hypothesized that correcting autonomic balance through NCC blockade reduces arrhythmogenicity, thereby improving survival among dnNRSF-Tg mice. Heart rate variability (HRV) is a widely accepted index of cardiac autonomic nervous system activity.[19] Earlier frequency domain analysis of HRV revealed that patients with severe heart failure show a progressive reduction in power in both the low-frequency (LF) and high-frequency (HF) ranges,[19] and that a reduction in the LF power range is a significant predictor of sudden cardiac death in patients with heart failure.[20] We used HRV as an index to evaluate cardiac autonomic function in WT and dnNRSF-Tg mice, and examined the effects of cilnidipine on HRV.[19] In mice, HRV predominantly correlates with parasympathetic activity.[21] As we showed previously, both the LF and HF powers averaged over 24 h in dnNRSF-Tg mice (LF, 1.228 ± 0.198; HF, 0.823 ± 0.186 m/s^2) were markedly lower than in WT mice (LF, 4.331 ± 0.706; HF, 2.412 ± 0.089 m/s^2), indicating a general reduction in parasympathetic activity in dnNRSF-Tg mice (*Figure 3A* and *B*). Cilnidipine dramatically increased the power in both the LF and HF ranges of HRV (LF, 3.308 ± 0.338; HF, 2.228 ± 0.283 m/s^2), whereas nitrendipine had little effect on HRV (LF, 0.538 ± 0.447; HF, 1.383 ± 0.57 m/s^2) (*Figure 3A* and *B*). We also found that urinary excretion of norepinephrine, which is indicative of the level of sympathetic nerve activity, was significantly higher in dnNRSF-Tg than in WT mice, and that norepinephrine excretion was significantly reduced only by cilnidipine (WT, 0.09 ± 0.02; Tg, 0.33 ± 0.04; Tg + cilnidipine, 0.15 ± 0.03; Tg + nitrendipine, 0.32 ± 0.1 μg/day) (*Figure 3C*).

We next used an implanted telemetric monitoring system to examine the effects of cilnidipine and nitrendipine on electrocardiographic parameters in dnNRSF-Tg mice. We found that only cilnidipine significantly suppressed the number of premature ventricular contractions (PVCs) in dnNRSF-Tg hearts (WT, 0 ± 0; dnNRSF-Tg, 502.66 ± 305.69; dnNRSF-Tg + cilnidipine, 1.0 ± 0.66; dnNRSF-Tg + nitrendipine, 326.17 ± 147.24 /h) (*Figure 3D*). More importantly, it dramatically reduced the number of episodes of ventricular tachycardia (VT) (WT, 0 ± 0; dnNRSF-Tg, 14.92 ± 4.95; dnNRSF-Tg + cilnidipine, 0.06 ± 0.06; dnNRSF-Tg + nitrendipine, 12.75 ± 5.16/h) (*Figure 3E* and Supplementary material online, *Figure S2A* and *B*). These lines of evidence suggest that by restoring autonomic nervous system balance, cilnidipine reduces the incidence of lethal arrhythmias in dnNRSF-Tg mice.

3.3 β-Adrenergic receptor blockade prevents lethal arrhythmias and sudden death in dnNRSF-Tg mice

To verify the importance of correcting autonomic nervous system imbalance for the prevention of lethal arrhythmias and sudden death in dnNRSF-Tg mice, irrespective of effects on structural remodelling, we examined the effects of treating these mice with a β-adrenergic receptor blocker. We administered a subpressor dose of the lipophilic β-adrenergic receptor blocker bisoprolol (1 mg/kg/day po) to WT and dnNRSF-Tg mice. Although systolic blood pressures did not differ between untreated control and bisoprolol-treated mice (untreated WT, 107.5 ± 1.6; WT + bisoprolol, 108.0 ± 1.2; untreated Tg, 98.6 ± 2.0; Tg + bisoprolol, 98.6 ± 1.7 mmHg) (*Figure 3F*), heart rates were significantly slower in bisoprolol-treated than in untreated WT and dnNRSF-Tg mice (untreated WT, 697.8 ± 8.3; WT + bisoprolol,

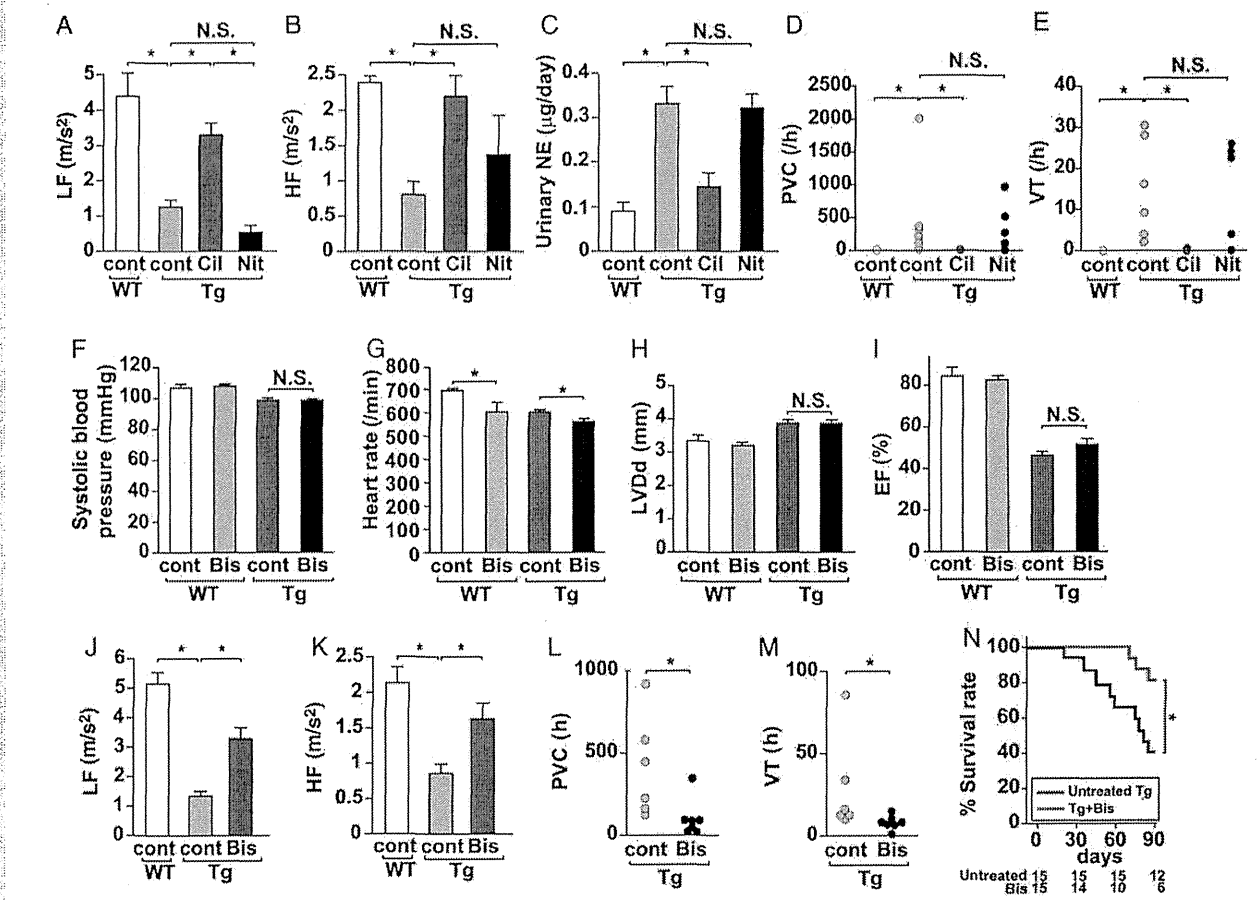

Figure 3 Cilnidipine restores cardiac autonomic nervous system balance and reduces arrhythmias in dnNRSF-Tg mice. (*A* and *B*) Average power of the LF (*A*) and HF (*B*) components of HRV recorded over a 24-h period in 20-week-old untreated WT (WT-cont), untreated Tg (Tg-cont), Cil-treated Tg (Tg-Cil), and Nit-treated (Tg-Nit) mice. $^*P < 0.05$. N.S.: not significant ($n = 5$ for WT, $n = 6$ for Tg-cont, $n = 8$ for Tg-Cil, and $n = 6$ for Tg-Nit). (*C*) Urinary norepinephrine (NE) levels in 20-week-old WT-cont, Tg-cont, Tg-Cil, and Tg-Nit mice. $^*P < 0.05$. N.S.: not significant ($n = 7$ for WT, $n = 7$ for Tg-cont, $n = 5$ for Tg-Cil, and $n = 4$ for Tg-Nit). (*D* and *E*) Numbers of PVC (*D*) and VT (*E*) recorded with a telemetry system in 20-week-old WT-cont, Tg-cont, Tg-Cil, and Tg-Nit mice are shown by dot plots. $^*P < 0.0083$, N.S.: not significant ($n = 5$ for WT-cont, $n = 6$ for Tg-cont, $n = 8$ for Tg-Cil, and $n = 6$ for Tg-Nit). (*F* and *G*) Systolic blood pressures (*F*) and heart rates (*G*) in 20-week-old untreated WT (WT-cont), bisoprolol (Bis)-treated WT (WT-Bis), untreated Tg (Tg-cont), and Bis-treated Tg (Tg-Bis) mice ($n = 4$ for WT-cont, $n = 3$ for WT-Bis, and $n = 5$ for Tg-cont and Tg-Bis). (*H* and *I*) LVDd (*H*) and EF (*I*) assessed echocardiographically in WT-cont, WT-Bis, Tg-cont, and Tg-Bis mice. $^*P < 0.05$. N.S.: not significant. ($n = 4$ for WT-cont, $n = 3$ for WT-Bis, and $n = 5$ for Tg-cont and Tg-Bis). (*J* and *K*) Average power of the LF (*J*) and HF (*K*) components of heart rate variability (HRV) recorded over a 24-h period in 20-week-old WT-cont, Tg-cont, and Tg-Bis mice. $^*P < 0.05$. N.S.: not significant ($n = 4$ for WT-cont, $n = 6$ for Tg-cont, and $n = 7$ for Tg-Bis). (*L* and *M*) Numbers of PVC (*L*) and VT (*M*) recorded with a telemetry system in 20-week-old Tg-cont and Tg-Bis mice are shown by dot plots. $^*P < 0.05$ ($n = 6$ for Tg-cont; $n = 7$ for Tg-Bis). ANOVA with post hoc Fisher's tests was used for analysis, except for numbers of arrhythmias (*D*, *E*, *L*, and *M*). Numbers of arrhythmias among the four groups were analyzed using Kruskal–Wallis non-parametric ANOVA followed by the Bonferroni correction (*D* and *E*). Numbers of arrhythmias between two groups were analyzed using non-parametric Mann–Whitney test (*L* and *M*). (*N*) Kaplan–Meyer survival curves for untreated Tg and Bis-treated Tg (Tg + Bis) over a 90-day drug administration period (from 12 to 25 weeks of age). Log-rank test was used for the survival analysis. $^*P < 0.05$ ($n = 15$ each). The numbers of mice alive in each group at the end of each period are shown at the bottom of the figure. All data except numbers of arrhythmias and survival curves are shown as means ± SEM.

604.7 ± 38.3; Tg, 601.6 ± 10.1; Tg + bisoprolol, 558.6 ± 12.0/min) (*Figure 3G*). At the dose tested, bisoprolol also did not affect cardiac systolic function assessed echocardiographically in dnNRSF-Tg mice [LVDd: WT, 3.3 ± 0.2; WT + bisoprolol, 3.2 ± 0.1; Tg, 3.9 ± 0.1; Tg + bisoprolol, 3.9 ± 0.1 mm and ejection fraction (EF): WT, 84.5 ± 4.0; WT + bisoprolol, 83.0 ± 1.5; Tg, 46.0 ± 1.6; Tg + bisoprolol, 51.5 ± 2.7%] (*Figure 3H* and *I*). On the other hand, bisoprolol significantly restored power in both the LF and HF ranges of HRV (LF: untreated WT, 5.19 ± 0.37; Tg, 1.36 ± 0.14; Tg + bisoprolol, 3.34 ± 0.39 m/s^2 and HF: untreated WT, 2.12 ± 0.24; Tg, 0.86 ± 0.12; Tg + bisoprolol, 1.62 ± 0.22 m/s^2) (*Figure 3J* and *K*) and reduced the incidence of PVCs and VTs in those mice (PVC: Tg, 408.3 ± 122.9; Tg + bisoprolol, 98.9 ± 42.2/h; VT: Tg, 28.2 ± 12.1; Tg + bisoprolol, 7.6 ± 1.7/h) (*Figure 3L* and *M*). As a result, bisoprolol significantly improved survival rates among dnNRSF-Tg mice (*Figure 3N*). These results strongly support our finding that imbalance of autonomic nervous system

activities is critically involved in the occurrence of sudden arrhythmic death in dnNRSF-Tg mice.

3.4 Genetic titration of NCC improves survival among dnNRSF-Tg mice

To further confirm the benefit of NCC inhibition for prevention of sudden death in dnNRSF-Tg mice, we next genetically titrated NCC expression by crossing dnNRSF-Tg mice with mice lacking *CACNA1B*, encoding the α1B subunit of NCC. Because the *CACNA1B*$^{-/-}$ genotype has a high incidence of early mortality from an as yet unknown cause, we compared the phenotypes of dnNRSF-Tg;*CACNA1B*$^{+/+}$ mice with those of dnNRSF-Tg;*CACNA1B*$^{+/-}$ mice, in which NCC expression is reduced to ~52.9% of that in dnNRSF-Tg;*CACNA1B*$^{+/+}$ mice (*Figure 4A*). The gross appearance of *CACNA1B*$^{+/-}$ mice is normal, and they show no early mortality. Systolic blood pressures in dnNRSF-Tg;*CACNA1B*$^{+/-}$ and dnNRSF-Tg;*CACNA1B*$^{+/+}$ mice did not significantly differ, but they were mildly lower than in control WT (*CACNA1B*$^{+/+}$) mice (WT, 101.25 ± 7.26; *CACNA1B*$^{+/-}$, 91.25 ± 2.78; dnNRSF-Tg, 92 ± 4.38; dnNRSF-Tg;*CACNA1B*$^{+/-}$, 89.25 ± 2.14 mmHg) (*Figure 4B*). Similarly, heart rates did not differ between dnNRSF-Tg;*CACNA1B*$^{+/+}$ and dnNRSF-Tg;*CACNA1B*$^{+/-}$ mice, although they were slower in dnNRSF-Tg;*CACNA1B*$^{+/+}$ than in control WT mice, as reported previously (WT, 632.25 ± 26.36; *CACNA1B*$^{+/-}$, 594 ± 33.39; dnNRSF-Tg, 515.25 ± 14.71; dnNRSF-Tg;*CACNA1B*$^{+/-}$, 521.5 ± 23.32/min) (*Figure 4C*).[8] Body weights were comparable between the two dnNRSF-Tg groups (WT, 31.08 ± 1.11; *CACNA1B*$^{+/-}$, 29.53 ± 1.37; dnNRSF-Tg; 28.86 ± 1.19; dnNRSF-Tg;*CACNA1B*$^{+/-}$, 27.41 ± 1.09 g) (*Figure 4D*), but heart-to-body weight ratios were higher in dnNRSF-Tg;*CACNA1B*$^{+/+}$ than in WT (*CACNA1B*$^{+/+}$) mice and were significantly lower in dnNRSF-Tg;*CACNA1B*$^{+/-}$ than in dnNRSF-Tg;*CACNA1B*$^{+/+}$ mice (WT, 4.44 ± 0.04; *CACNA1B*$^{+/-}$, 4.51 ± 0.14; dnNRSF-Tg; 5.68 ± 0.21; dnNRSF-Tg;*CACNA1B*$^{+/-}$, 4.86 ± 0.18 mg/g) (*Figure 4E*). Lung-to-body weight ratios were comparable between the two dnNRSF-Tg groups (WT, 5.06 ± 0.22; *CACNA1B*$^{+/-}$, 4.68 ± 0.96; dnNRSF-Tg; 5.41 ± 0.09; dnNRSF-Tg; *CACNA1B*$^{+/-}$, 5.52 ± 0.26 mg/g) (*Figure 4F*). Echocardiographic analysis showed that left ventricular diastolic dimension (LVDd) was higher in dnNRSF-Tg;*CACNA1B*$^{+/+}$ than in WT mice, whereas EF was lower in dnNRSF-Tg;*CACNA1B*$^{+/+}$ than in WT mice, as was reported previously (*Figure 5A* and *B*).[8] In addition, LVDd was lower and EF was higher in dnNRSF-Tg;*CACNA1B*$^{+/-}$ than in dnNRSF-Tg;*CACNA1B*$^{+/+}$ mice (*Figure 5A* and *B* and *Table 1*).

Histological analysis revealed no significant difference between dnNRSF-Tg;*CACNA1B*$^{+/+}$ and dnNRSF-Tg;*CACNA1B*$^{+/-}$ mice, although %fibrotic area showed a trend towards being smaller in dnNRSF-Tg;*CACNA1B*$^{+/-}$ than in dnNRSF-Tg;*CACNA1B*$^{+/+}$ mice (*Figure 5C* and *D*). Expression of the fibrosis-related genes *Col1a1*, *Col3a1*, and *FN1* did not significantly differ between dnNRSF-Tg;*CACNA1B*$^{+/+}$ and dnNRSF-Tg;*CACNA1B*$^{+/-}$ mice (see Supplementary material online, *Figure S3A–C*), though there was a significant difference in the expression of *ANP* and *SERCA2* between these two genotypes (*Figure 5E* and *F*). Genetic reduction in *CACNA1B* also significantly affected expression of *CACNA1H* and *HCN2*, but not *HCN4*, in dnNRSF-Tg ventricles (see Supplementary material online, *Figure S3D–F*). All of these data demonstrate that genetic reduction of *CACNA1B* tends to ameliorate impaired cardiac function and pathological remodelling in dnNRSF-Tg mice. Furthermore, survival among dnNRSF-Tg;*CACNA1B*$^{+/-}$ mice was dramatically and significantly better than among control dnNRSF-Tg;*CACNA1B*$^{+/+}$ mice (*Figure 6A*), demonstrating that reduction of NCC prevents sudden arrhythmic death in dnNRSF-Tg mice.

3.5 Reducing *CACNA1B* expression improves autonomic function and decreases the occurrence of arrhythmias in dnNRSF-Tg mice

We also assessed autonomic nervous system activity in dnNRSF-Tg;*CACNA1B*$^{+/-}$ and dnNRSF-Tg;*CACNA1B*$^{+/+}$ mice. In HRV analyses, the reductions in LF and HF power otherwise seen in dnNRSF-Tg;*CACNA1B*$^{+/+}$ mice (LF, 1.288 ± 0.16; HF, 1.168 ± 0.108 m/s^2) were significantly ameliorated in dnNRSF-Tg;*CACNA1B*$^{+/-}$ mice (LF, 3.54 ± 0.47; HF, 3.075 ± 0.468 m/s^2), indicating a restoration of parasympathetic activity through reduction of NCC function (*Figure 6B* and *C*). In addition, we found that the increase in urinary excretion of norepinephrine seen in dnNRSF-Tg;*CACNA1B*$^{+/+}$ mice (0.428 ± 0.07 μg/day) was significantly ameliorated in dnNRSF-Tg;*CACNA1B*$^{+/-}$ mice (0.154 ± 0.05 μg/day) (*Figure 6D*). Finally, evaluation of arrhythmicity revealed that the incidences of both PVCs and VT were significantly lower in dnNRSF-Tg;*CACNA1B*$^{+/-}$ than in dnNRSF-Tg;*CACNA1B*$^{+/+}$ mice (PVC: WT, 0 ± 0; *CACNA1B*$^{+/-}$, 0 ± 0; dnNRSF-Tg, 239.08 ± 27.93; dnNRSF-Tg;*CACNA1B*$^{+/-}$, 3.21 ± 3.21 and VT: WT, 0 ± 0; *CACNA1B*$^{+/-}$, 0 ± 0; dnNRSF-Tg, 41.3 ± 12.69; dnNRSF-Tg;*CACNA1B*$^{+/-}$, 0.36 ± 0.36/h) (*Figure 6E* and *F*). These results demonstrate that genetic titration of *CACNA1B*, encoding NCC, corrected an imbalance between sympathetic and parasympathetic nervous system activities, which, at least in part, contributes to reducing malignant arrhythmias in dnNRSF-Tg mice in a manner similar to pharmacological NCC blockade.

4. Discussion

Autonomic dysregulation leading to increased sympathetic nerve activity and reduced parasympathetic nerve activity is reportedly associated with the increased arrhythmicity seen in patients with chronic heart failure.[2,22,23] NCCs play a major role in the release of norepinephrine at sympathetic nerve terminals.[7,24] Consequently, mice lacking *CACNA1B*, the gene encoding the α1 subunit of NCCs, exhibit a significantly impaired positive inotropic response.[7] In the present study, we found that pharmacological blockade of NCCs or their genetic titration improved the balance between sympathetic and parasympathetic nerve activities and prevented the sudden death and arrhythmicity otherwise seen in dnNRSF-Tg mice, a mouse model of sudden arrhythmic death associated with cardiac dysfunction.[8] The mode of death in these model mice is sudden and without overt oedema, pleural effusion, or apparent lung congestion, and all the telemetry data obtained at the time of death indicate VT/VF to be the cause.[8] Moreover, in an earlier study, we found that systemic administration of isoproterenol induced VT more frequently in dnNRSF-Tg than in WT mice.[11] Conversely, administration of a β-blocker led to a significant reduction in the incidence of sudden death among dnNRSF-Tg mice under conditions in which cardiac systolic function and remodelling were not affected (*Figure 3H–N*). These findings suggest that NCC blockade or genetic titration of NCC reduces the likelihood of sudden arrhythmic death, thereby improving survival.

Pharmacological interventions that reduce cardiac sympathetic activity have been shown to protect against arrhythmias,[25] while

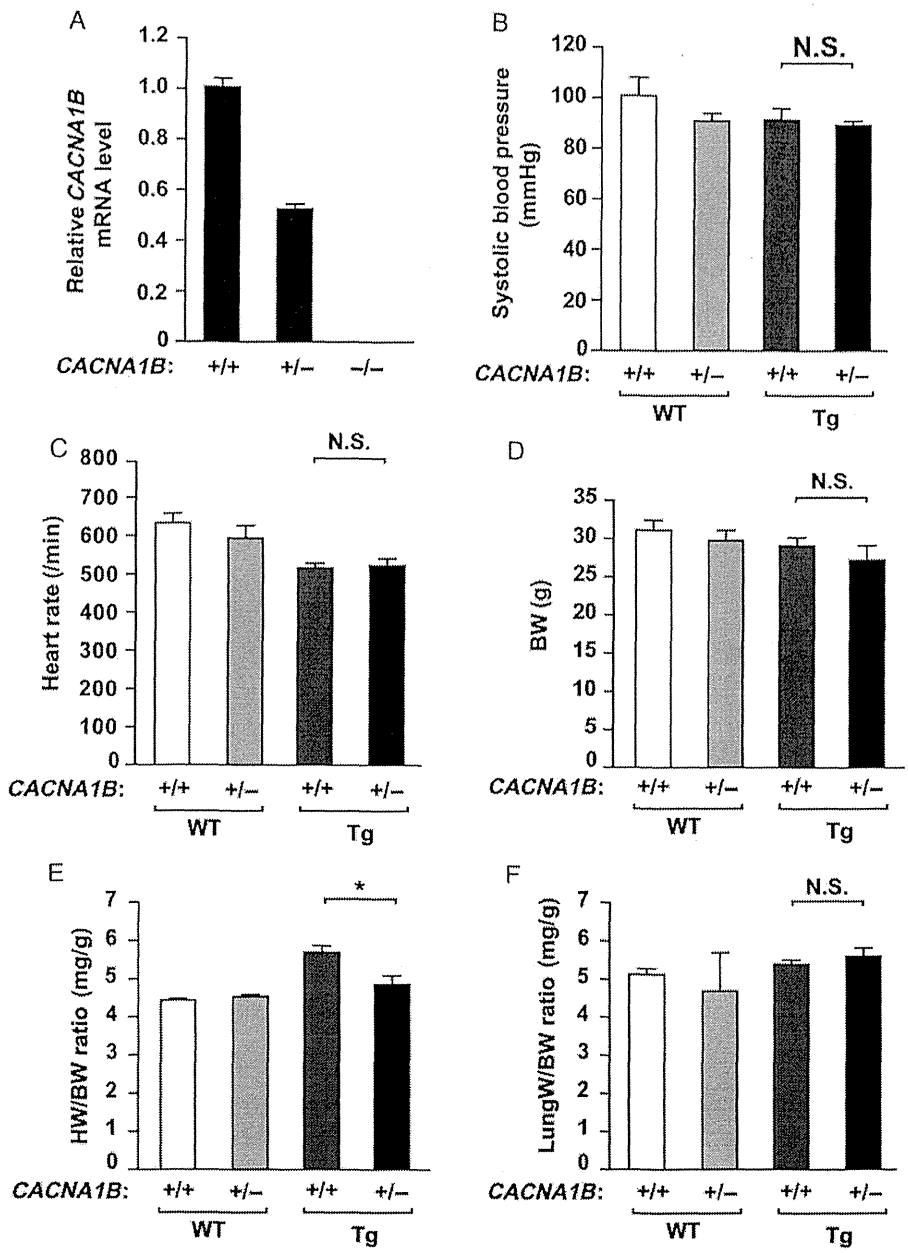

Figure 4 Effects of genetic titration of *CACNA1B* on hemodynamics and heart size in WT and dnNRSF-Tg mice. (*A*) CACNA1B mRNA expression in brains from 8-week-old CACNA1B$^{+/+}$, CACNA1B$^{+/-}$, and CACNA1B$^{-/-}$ mice; the level in CACNA1B$^{+/+}$ brain was assigned a value of 1.0. (*B* and *C*) Systolic blood pressures (*B*) and heart rates (*C*) in 20-week-old CACNA1B$^{+/+}$, CACNA1B$^{+/-}$, dnNRSF-Tg;CACNA1B$^{+/+}$, and dnNRSF-Tg;CACNA1B$^{+/-}$ mice. N.S.: not significant ($n = 4$ each). (*D*, *E*, and *F*) body weights (BW)(*D*), heart-to-body weight ratios (HW/BW) (*E*), and lung-to-body weight ratios (LungW/BW) (*F*) in 20-week-old CACNA1B$^{+/+}$, CACNA1B$^{+/-}$, dnNRSF-Tg;CACNA1B$^{+/+}$, and dnNRSF-Tg;CACNA1B$^{+/-}$ mice. *$P < 0.05$. N.S.: not significant. (BW and HW/BW: $n = 4$ for CACNA1B$^{+/+}$, $n = 6$ for CACNA1B$^{+/-}$, $n = 5$ for dnNRSF-Tg;CACNA1B$^{+/+}$, and $n = 7$ for dnNRSF-Tg;CACNA1B$^{+/-}$; LungW/BW: $n = 4$ for CACNA1B$^{+/+}$, $n = 6$ for CACNA1B$^{+/-}$ and dnNRSF-Tg;CACNA1B$^{+/-}$, and $n = 5$ for dnNRSF-Tg;CACNA1B$^{+/+}$). ANOVA with post hoc Fisher's tests was used for analysis. All data are shown as means ± SEM.

interventions that stimulate cardiac sympathetic activity provoke malignant arrhythmias.[2,26] In patients with heart failure, β-adrenoreceptor blockade reduces the incidence of sudden death;[27,28] however, β-blockers are not completely protective, and mortality remains high among patients with cardiac dysfunction, despite optimal β-blocker therapy.[27,28] It is therefore necessary to find other approaches to modulate sympathetic or parasympathetic activity. In that context, a clinical trial testing the effect of central modulation of sympathetic activity using moxonidine SR in patients with heart failure was terminated early due to an increase in mortality and morbidity in patients receiving the drug.[29] Thus, strong central inhibition of the sympathetic nervous system through imidazoline receptor stimulation appears not to

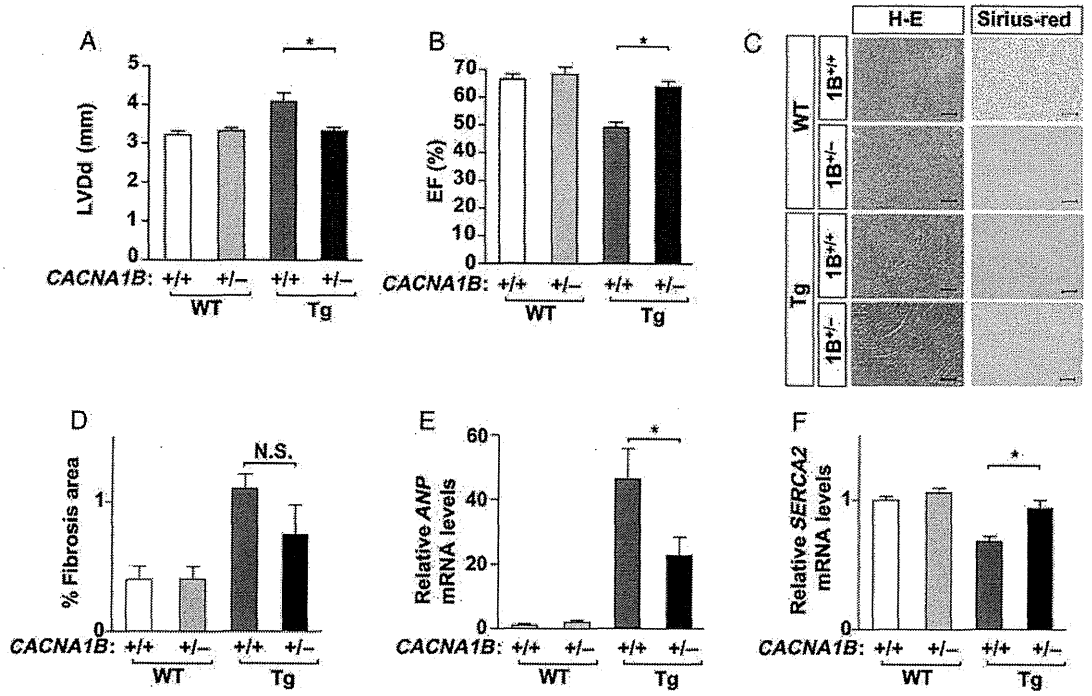

Figure 5 Effect of genetic titration of *CACNA1B* on cardiac structure and function in dnNRSF-Tg mice. (*A* and *B*) LVDd and EF assessed echocardiographically in 20-week-old CACNA1B$^{+/+}$, CACNA1B$^{+/-}$, CACNA1B$^{+/+}$;dnNRSF-Tg and CACNA1B$^{+/-}$;dnNRSF-Tg mice. *$P < 0.05$. ($n = 13$ for CACNA1B$^{+/+}$, $n = 14$ for CACNA1B$^{+/-}$, $n = 11$ for dnNRSF-Tg;CACNA1B$^{+/+}$, and $n = 15$ for dnNRSF-Tg;CACNA1B$^{+/-}$). (*C*) Histology of hearts from 20-week-old CACNA1B$^{+/+}$, CACNA1B$^{+/-}$, dnNRSF-Tg;CACNA1B$^{+/+}$ and dnNRSF-Tg;CACNA1B$^{+/-}$ mice. H-E, haematoxylin-eosin staining; Sirius-red, Sirius-red staining. Scale bars = 100 μm. (*D*) %Fibrotic area in the indicated groups ($n = 4$ for CACNA1B$^{+/+}$, $n = 6$ for CACNA1B$^{+/-}$, $n = 5$ for dnNRSF-Tg;CACNA1B$^{+/+}$, and $n = 7$ for dnNRSF-Tg;CACNA1B$^{+/-}$). N.S. not significant. (*E* and *F*) Relative levels of ANP and SERCA2 mRNA in cardiac ventricles from 20-week-old CACNA1B$^{+/+}$, CACNA1B$^{+/-}$, dnNRSF-Tg;CACNA1B$^{+/+}$ and dnNRSF-Tg;CACNA1B$^{+/-}$ mice ($n = 4$ for CACNA1B$^{+/+}$, $n = 6$ for CACNA1B$^{+/-}$, and $n = 5$ for dnNRSF-Tg;CACNA1B$^{+/+}$ and dnNRSF-Tg;CACNA1B$^{+/-}$); levels in CACNA1B$^{+/+}$ ventricles were assigned a value of 1.0. *$P < 0.05$. ANOVA with post hoc Fisher's tests was used for analysis. All data are shown as means ± SEM.

protect against lethal arrhythmias. NCCs are localized at peripheral sympathetic nerve terminals, where they regulate the release of neurotransmitters (e.g. catecholamines), thereby modulating sympathetic activity.[4–6] Our findings suggest that, by correcting their autonomic dysregulation, NCC blockade could be an effective approach to preventing sudden arrhythmic death in patients with heart failure.

Cilnidipine failed to prevent the decline in cardiac function in dnNRSF-Tg mice, whereas genetic titration tended to ameliorate the adverse cardiac remodelling and cardiac dysfunction seen in dnNRSF-Tg mice (*Figures 2A–H*, *4E*, and *5A–F* and *Table 1*). The reasons for the difference in the effects on cardiac function between cilnidipine and genetic titration of NCCs remain unclear at present. It may be that cilnidipine's ability to block L-type Ca^{2+} channels has a detrimental effect on cardiac function, as L-type Ca^{2+} channel blockers can adversely affect the progression of heart failure.[30] Other possibilities are that the relatively low dose of cilnidipine used in this study was not sufficient to prevent the progression of cardiac dysfunction, though it did prevent lethal arrhythmias, or that the NCC inhibition achieved in *CACNA1B*$^{+/-}$ mice was more prolonged and more stable than that achieved with cilnidipine, which was not started until the mice were 8 weeks of age. The effects on NCCs expressed in the central nervous system could also differ between cilnidipine and genetic titration, as cilnidipine has little ability to cross the blood–brain barrier.[31] These differences suggest the underlying mechanisms involved in the reduced incidence of lethal arrhythmias, and the prolonged survival differ somewhat between cilnidipine treatment and genetic titration of *CACNA1B* in this study. Cilnidipine treatment, which improved autonomic imbalance and reduced lethal arrhythmias without affecting cardiac remodelling, mainly suppressed the triggering of lethal arrhythmias induced by autonomic imbalance. On the other hand, genetic titration of *CACNA1B*, which improved autonomic imbalance and also tended to prevent adverse cardiac remodelling, suppressed lethal arrhythmias and improved survival in two ways: it inhibited the triggering of arrhythmias and also suppressed the generation of arrhythmogenic substrates. In both cases, correcting the autonomic imbalance associates with a reduction in the incidence of sudden death attributable to lethal arrhythmias in dnNRSF-Tg. However, because it is not possible to completely exclude the possibility that some dnNRSF-Tg mice (especially older mice) die due to congestive heart failure, irrespective of arrhythmias, there is a possibility that genetic deletion of NCC may also prevent this mode of death in addition to sudden arrhythmic death in dnNRSF-Tg mice through suppression of excessive sympathetic activity.

In the present study, both pharmacological blockade of NCCs and their genetic titration not only repressed sympathetic activity, as demonstrated by a reduction in urinary norepinephrine levels, but also restored parasympathetic activity, as indicated by HRV analyses. The precise

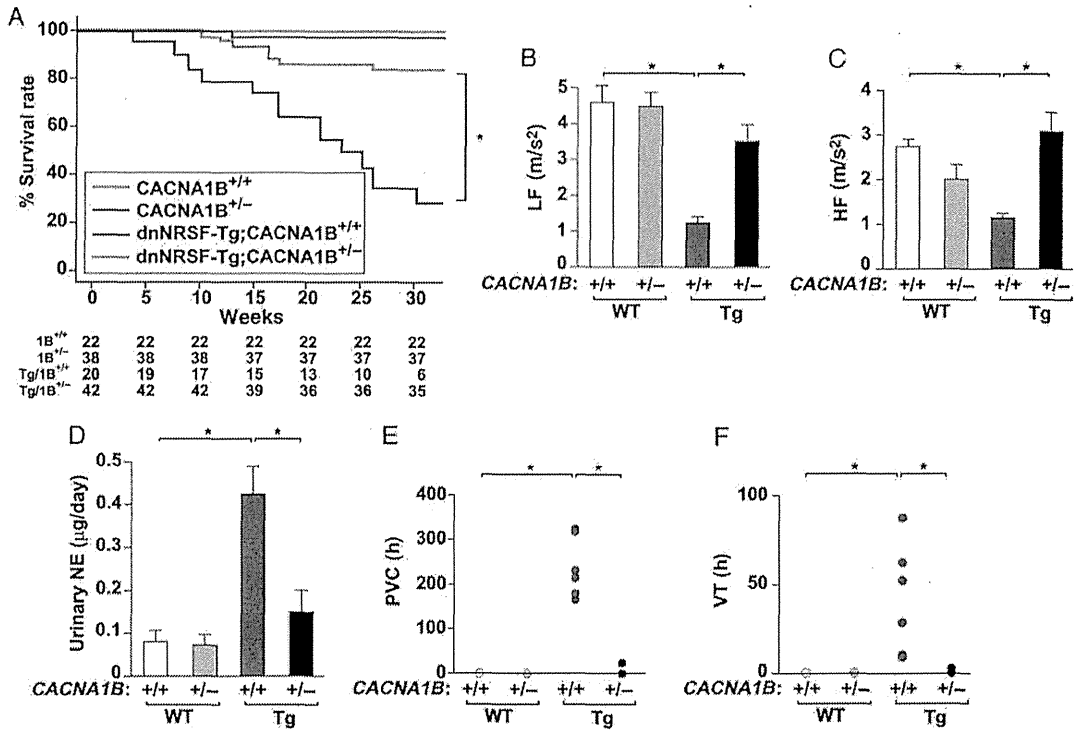

Figure 6 Genetic titration of *CACNA1B* restores cardiac autonomic nervous system balance and reduces arrhythmias in dnNRSF-Tg mice. (*A*) Kaplan–Meyer survival curves for CACNA1B$^{+/+}$ (1B$^{+/+}$), CACNA1B$^{+/-}$ (1B$^{+/-}$), dnNRSF-Tg;CACNA1B$^{+/+}$ (Tg/1B$^{+/+}$), and dnNRSF-Tg;CACNA1B$^{+/-}$ (Tg/1B$^{+/-}$) mice. Curves cover the span from birth to 32 weeks of age. Log-rank test was used for analysis. *$P < 0.05$ ($n = 22$ for CACNA1B$^{+/+}$, $n = 38$ for CACNA1B$^{+/-}$, $n = 20$ for dnNRSF-Tg;CACNA1B$^{+/+}$, and $n = 42$ for dnNRSF-Tg;CACNA1B$^{+/-}$). The numbers of mice alive in each group at the end of each period are shown at the bottom of the figure. (*B* and *C*) Average power of the LF (*B*) and HF (*C*) components of heart rate variability (HRV) recorded over a 24-h period in 20-week-old CACNA1B$^{+/+}$, CACNA1B$^{+/-}$, dnNRSF-Tg;CACNA1B$^{+/+}$, and dnNRSF-Tg;CACNA1B$^{+/-}$ mice. *$P < 0.05$ ($n = 5$ for CACNA1B$^{+/+}$, $n = 7$ for CACNA1B$^{+/-}$, $n = 6$ for dnNRSF-Tg;CACNA1B$^{+/+}$, and $n = 7$ for dnNRSF-Tg;CACNA1B$^{+/-}$). (*D*) Urinary norepinephrine (NE) levels in 20-week-old CACNA1B$^{+/+}$, CACNA1B$^{+/-}$, dnNRSF-Tg;CACNA1B$^{+/+}$, and dnNRSF-Tg;CACNA1B$^{+/-}$ mice. *$P < 0.05$ ($n = 5$ for CACNA1B$^{+/-}$, $n = 6$ for CACNA1B$^{+/+}$, dnNRSF-Tg;CACNA1B$^{+/+}$, and dnNRSF-Tg;CACNA1B$^{+/-}$). (*E* and *F*) Numbers of PVC (*E*) and VT (*F*) recorded using a telemetry system in 20-week-old CACNA1B$^{+/+}$, CACNA1B$^{+/-}$, dnNRSF-Tg;CACNA1B$^{+/+}$, and dnNRSF-Tg;CACNA1B$^{+/-}$ mice are shown by dot plot. *$P < 0.0083$ ($n = 5$ for CACNA1B$^{+/+}$, $n = 7$ for CACNA1B$^{+/-}$, $n = 6$ for dnNRSF-Tg;CACNA1B$^{+/+}$, and $n = 7$ for dnNRSF-Tg;CACNA1B$^{+/-}$). All data in *B*–*D* are shown as means ± SEM. ANOVA with post hoc Fisher's tests was used for analysis, except for numbers of arrhythmias (*E* and *F*). Numbers of arrhythmias were analyzed using Kruskal–Wallis non-parametric ANOVA followed by the Bonferroni correction.

mechanism by which NCC inhibition improves parasympathetic activity is not clear at present. However, accumulating data indicate the sympathetic and parasympathetic nervous systems interact via several mechanisms at both the central and peripheral levels of the neuraxis.[32] NCC inhibition-induced reductions in sympathetic activity may affect these interactions, ameliorating the reduction in parasympathetic activity, as was observed in dnNRSF-Tg mice. In humans, cilnidipine reportedly enhances parasympathetic activity in hypertensive patients while exerting a concomitant sympathoinhibitory effect.[12,13] Moreover, there is now much evidence showing the anti-arrhythmic effects of parasympathetic nervous activation. This suggests that, in addition to a reduction in sympathetic activity, an increase in parasympathetic activity likely contributes to the protective effects of NCC inhibition observed in this study.[27] Although further investigation is necessary, our study suggests that agents able to selectively block NCCs could be clinically useful for the prevention of sudden arrhythmic death in patients with heart failure.

Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

Acknowledgements

We thank Ms Yukari Kubo for her excellent secretarial work and Ms Aoi Fujishima, Ms Akiko Abe, Mr Miku Ohya, and Ms Mizuho Takemura for their excellent technical support.

Conflict of interest: none declared.

Funding

This research was supported by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (23390210, 24659386 to K.K., 24591095 to H.K., 22590810 to Y.N., 21229013 to N.K.); the Japanese Ministry of Health, Labor and Welfare (N.K.); the Japan Foundation for Applied Enzymology (K.K.); the UBE foundation (K.K.); the Ichiro Kanehara

Foundation (K.K.); the Takeda Science Foundation (K.K.); the Hoh-ansha Foundation (K.K.); the SENSHIN Medical Research Foundation (K.K.).

References

1. Tomaselli GF, Marban E. Electrophysiological remodeling in hypertrophy and heart failure. *Cardiovasc Res* 1999;**42**:270–283.
2. Anderson KP. Sympathetic nervous system activity and ventricular tachyarrhythmias: recent advances. *Ann Noninvasive Electrocardiol* 2003;**8**:75–89.
3. Chen PS, Chen LS, Cao JM, Sharifi B, Karagueuzian HS, Fishbein MC. Sympathetic nerve sprouting, electrical remodeling and the mechanisms of sudden cardiac death. *Cardiovasc Res* 2001;**50**:409–416.
4. Mori Y, Nishida M, Shimizu S, Ishii M, Yoshinaga T, Ino M, Sawada K, Niidome T. Ca(2+) channel alpha(1B) subunit (Ca(V) 2.2) knockout mouse reveals a predominant role of N-type channels in the sympathetic regulation of the circulatory system. *Trends Cardiovasc Med* 2002;**12**:270–275.
5. Himing LD, Fox AP, McCleskey EW, Olivera BM, Thayer SA, Miller RJ, Tsien RW. Dominant role of N-type Ca2+ channels in evoked release of norepinephrine from sympathetic neurons. *Science* 1988;**239**:57–61.
6. Fujita Y, Mynlieff M, Dirksen RT, Kim MS, Niidome T, Nakai J, Friedrich T, Iwabe N, Miyata T, Furuichi T, Furutama D, Mikoshiba K, Mori Y, Beam KG. Primary structure and functional expression of the omega-conotoxin-sensitive N-type calcium channel from rabbit brain. *Neuron* 1993;**10**:585–598.
7. Ino M, Yoshinaga T, Wakamori M, Miyamoto N, Takahashi E, Sonoda J, Kagaya T, Oki T, Nagasu T, Nishizawa Y, Tanaka I, Imoto K, Aizawa S, Koch S, Schwartz A, Niidome T, Sawada K, Mori Y. Functional disorders of the sympathetic nervous system in mice lacking the alpha 1B subunit (Cav 2.2) of N-type calcium channels. *Proc Natl Acad Sci USA* 2001;**98**:5323–5328.
8. Kuwahara K, Saito Y, Takano M, Arai Y, Yasuno S, Nakagawa Y, Takahashi N, Adachi Y, Takemura G, Horie M, Miyamoto Y, Morisaki T, Kuratomi S, Noma A, Fujiwara H, Yoshimasa Y, Kinoshita H, Kawakami R, Kishimoto I, Nakanishi M, Usami S, Harada M, Nakao K. NRSF regulates the fetal cardiac gene program and maintains normal cardiac structure and function. *EMBO J* 2003;**22**:6310–6321.
9. Kuwabara Y, Kuwahara K, Takano M, Kinoshita H, Arai Y, Yasuno S, Nakagawa Y, Igata S, Usami S, Minami T, Yamada Y, Nakao K, Yamada C, Shibata J, Nishikimi T, Ueshima K, Nakao K. Increased expression of HCN channels in the ventricular myocardium contributes to enhanced arrhythmicity in mouse failing hearts. *J Am Heart Assoc* 2013;**2**: e000150.
10. Takano M, Kinoshita H, Shioya T, Itoh M, Nakao K, Kuwahara K. Pathophysiological remodeling of mouse cardiac myocytes expressing dominant negative mutant of neuron restrictive silencing factor. *Circ J* 2010;**74**:2712–2719.
11. Kinoshita H, Kuwahara K, Takano M, Arai Y, Kuwabara Y, Yasuno S, Nakagawa Y, Nakanishi M, Harada M, Fujiwara M, Murakami M, Ueshima K, Nakao K. T-type Ca2+ channel blockade prevents sudden death in mice with heart failure. *Circulation* 2009; **120**:743–752.
12. Kishi T, Hirooka Y, Konno S, Sunagawa K. Cilnidipine inhibits the sympathetic nerve activity and improves baroreflex sensitivity in patients with hypertension. *Clin Exp Hypertens* 2009;**31**:241–249.
13. Ogura C, Ono K, Miyamoto S, Ikai A, Mitani S, Sugimoto N, Tanaka S, Fujita M. L/T-type and L/N-type calcium-channel blockers attenuate cardiac sympathetic nerve activity in patients with hypertension. *Blood Press* 2012;**21**:367–371.
14. Egashira N, Okuno R, Abe M, Matsushita M, Mishima K, Iwasaki K, Oishi R, Nishimura R, Matsumoto Y, Fujiwara M. Calcium-channel antagonists inhibit marble-burying behavior in mice. *J Pharmacol Sci* 2008;**108**:140–143.
15. Lei B, Nakano D, Fujisawa Y, Liu Y, Hitomi H, Kobori H, Mori H, Masaki T, Asanuma K, Tomino Y, Nishiyama A. N-type calcium channel inhibition with cilnidipine elicits glomerular podocyte protection independent of sympathetic nerve inhibition. *J Pharmacol Sci* 2012;**119**:359–367.
16. Uneyama H, Uchida H, Konda T, Yoshimoto R, Akaike N. Selectivity of dihydropyridines for cardiac L-type and sympathetic N-type Ca2+ channels. *Eur J Pharmacol* 1999;**373**: 93–100.
17. Fujii S, Kameyama K, Hosono M, Hayashi Y, Kitamura K. Effect of cilnidipine, a novel dihydropyridine Ca++-channel antagonist, on N-type Ca++ channel in rat dorsal root ganglion neurons. *J Pharmacol Exp Ther* 1997;**280**:1184–1191.
18. Johnson R, Gamblin RJ, Ooi L, Bruce AW, Donaldson IJ, Westhead DR, Wood IC, Jackson RM, Buckley NJ. Identification of the REST regulon reveals extensive transposable element-mediated binding site duplication. *Nucleic Acids Res* 2006;**34**:3862–3877.
19. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Eur Heart J* 1996;**17**:354–381.
20. La Rovere MT, Pinna GD, Maestri R, Mortara A, Capomolla S, Febo O, Ferrari R, Franchini M, Gnemmi M, Opasich C, Riccardi PG, Traversi E, Cobelli F. Short-term heart rate variability strongly predicts sudden cardiac death in chronic heart failure patients. *Circulation* 2003;**107**:565–570.
21. Just A, Faulhaber J, Ehmke H. Autonomic cardiovascular control in conscious mice. *Am J Physiol Regul Integr Comp Physiol* 2000;**279**:R2214–e002221.
22. Brack KE, Winter J, Ng GA. Mechanisms underlying the autonomic modulation of ventricular fibrillation initiation-tentative prophylactic properties of vagus nerve stimulation on malignant arrhythmias in heart failure. *Heart Fail Rev* 2013;**18**:389–408.
23. Schwartz PJ, La Rovere MT, Vanoli E. Autonomic nervous system and sudden cardiac death. Experimental basis and clinical observations for post-myocardial infarction risk stratification. *Circulation* 1992;**85**:I77–I91.
24. Molderings GJ, Likungu J, Gothert M. N-Type calcium channels control sympathetic neurotransmission in human heart atrium. *Circulation* 2000;**101**:403–407.
25. Billman GE. Cardiac autonomic neural remodeling and susceptibility to sudden cardiac death: effect of endurance exercise training. *Am J Physiol Heart Circ Physiol* 2009;**297**: H1171–H1193.
26. Volders PG. Novel insights into the role of the sympathetic nervous system in cardiac arrhythmogenesis. *Heart Rhythm* 2010;**7**:1900–1906.
27. Packer M, Coats AJ, Fowler MB, Katus HA, Krum H, Mohacsi P, Rouleau JL, Tendera M, Castaigne A, Roecker EB, Schultz MK, DeMets DL. Effect of carvedilol on survival in severe chronic heart failure. *N Engl J Med* 2001;**344**:1651–1658.
28. The Cardiac Insufficiency Bisoprolol Study II (CIBIS-II): a randomised trial. *Lancet* 1999; **353**:9–13.
29. Cohn JN, Pfeffer MA, Rouleau J, Sharpe N, Swedberg K, Straub M, Wiltse C, Wright TJ. Adverse mortality effect of central sympathetic inhibition with sustained-release moxonidine in patients with heart failure (MOXCON). *Eur J Heart Fail* 2003;**5**:659–667.
30. Mahe I, Chassany O, Grenard AS, Caulin C, Bergmann JF. Defining the role of calcium channel antagonists in heart failure due to systolic dysfunction. *Am J Cardiovasc Drugs* 2003;**3**:33–41.
31. Watanabe K, Dozen M, Hayashi Y. Effect of cilnidipine (FRC-8653) on autoregulation of cerebral blood flow. *Nihon Yakurigaku Zasshi* 1995;**106**:393–399.
32. Ondicova K, Mravec B. Multilevel interactions between the sympathetic and parasympathetic nervous systems: a minireview. *Endocr Regul* 2010;**44**:69–75.

ORIGINAL ARTICLE

Extensive late gadolinium enhancement on cardiovascular magnetic resonance predicts adverse outcomes and lack of improvement in LV function after steroid therapy in cardiac sarcoidosis

Takayuki Ise,[1] Takuya Hasegawa,[1] Yoshiaki Morita,[2] Naoaki Yamada,[2] Akira Funada,[1] Hiroyuki Takahama,[1] Makoto Amaki,[1] Hideaki Kanzaki,[1] Hideo Okamura,[1] Shiro Kamakura,[1] Wataru Shimizu,[1] Toshihisa Anzai,[1] Masafumi Kitakaze[1,3]

[1]Department of Cardiovascular Medicine, National Cerebral and Cardiovascular Center, Osaka, Japan
[2]Department of Radiology, National Cerebral and Cardiovascular Center, Osaka, Japan
[3]Department of Clinical Research and Development, National Cerebral and Cardiovascular Center, Osaka, Japan

Correspondence to
Dr Takuya Hasegawa, Department of Cardiovascular Medicine National Cerebral and Cardiovascular Center, Osaka, Japan 5-7-1 Fujishiro-dai, Suita, Osaka 565-8565, Japan; hasegawa@hsp.ncvc.go.jp

Received 31 October 2013
Revised 17 April 2014
Accepted 22 April 2014
Published Online First 14 May 2014

ABSTRACT

Background Gadolinium-enhanced cardiovascular magnetic resonance is an emerging tool for the diagnosis of cardiac sarcoidosis (CS); however, the correlations between extent of late gadolinium enhancement (LGE) and efficacy of steroid therapy and adverse outcomes in patients with CS remain unclear.

Objective We aimed to clarify the prognostic impact of extent of LGE in patients with CS.

Methods Before the start of steroid therapy, 43 consecutive LGE-positive patients with CS were divided into two groups based on the extent of LGE by a median value: small-extent LGE (LGE mass <20% of LV mass; n=21) and large-extent LGE (LGE mass ≥20% of LV mass; n=22). We examined the correlations between extent of LGE and outcomes after steroid therapy.

Results Among the 6 patients who died from heart disorders, 11 patients who were hospitalised because of heart failure and 6 patients who suffered life-threatening arrhythmia during the follow-up period, large-extent LGE predicted higher incidences of cardiac mortality and hospitalisation for heart failure. Multivariate Cox regression analysis showed that large-extent LGE was independently associated with combined adverse outcomes including cardiac death, hospitalisation for heart failure, and life-threatening arrhythmias. In the small-extent LGE group, LV end-diastolic volume index significantly decreased and LVEF significantly increased after steroid therapy, whereas in the large-extent LGE group, neither LV volume nor LVEF changed substantially.

Conclusions Large-extent LGE correlates with absence of LV functional improvement and high incidence of adverse outcomes in patients with CS after steroid therapy.

INTRODUCTION

Sarcoidosis is a multisystem granulomatous disorder of unknown cause with symptomatic myocardial involvement in up to 7% of affected patients.[1–3] Although it is generally associated with a low mortality rate, concomitant cardiac involvement worsen its prognosis.[4 5] Therefore, detection of myocardial involvement is critical for management of patients with sarcoidosis. In patients with sarcoidosis, gadolinium-enhanced cardiovascular magnetic resonance (CMR) is a useful diagnostic tool to qualitatively detect myocardial involvement.[6–8] In most patients with cardiac sarcoidosis (CS), late gadolinium enhancement (LGE) is typically localised in the basal and lateral segments of the LV wall or epicardium, which does not fit any specific coronary perfusion area.[9] LGE in CMR has been reported to reflect myocardial fibrosis and granulomatous inflammation in patients with CS.[7] It has also been reported that the presence of myocardial LGE can predict adverse events in patients with systemic sarcoidosis.[7 10] However, the prognostic impact of the extent of LGE has not been fully investigated. In this study, we examined the correlations between the extent of LGE and adverse outcomes, as well as the efficacy of steroid therapy in patients with CS.

METHODS

Study patients

Medical records were screened to identify patients diagnosed with CS in our institution from May 2000 to May 2012. CS was diagnosed according to the guidelines of the Specific Diffuse Pulmonary Disease Research Group, Sarcoidosis Division (Japanese Ministry of Health and Welfare).[11] In brief, CS was diagnosed on the basis of histological findings or clinical findings. Histological diagnosis of CS was confirmed when histological analysis of endomyocardial biopsy specimens demonstrated epithelioid granuloma without caseating granulomas. Clinical diagnosis of CS was confirmed by the presence of an electrocardiographic (ECG) abnormality suggesting myocardial injury, and at least one of the following items: abnormal wall motion, regional wall thinning, or dilatation of the LV; perfusion defect on thallium-201 myocardial scintigraphy or abnormal accumulation by gallium-67-citrate scintigraphy or technetium-99m-pyrophosphatemyocardial scintigraphy; abnormal intracardiac pressure, low cardiac output, or depressed LVEF; and interstitial fibrosis or cellular infiltration over moderate grade even if the findings were non-specific. All patients underwent coronary angiography, and no significant coronary artery stenosis was noted. All baseline characteristics, including CMR data, were collected

To cite: Ise T, Hasegawa T, Morita Y, *et al*. *Heart* 2014;**100**:1165–1172.