Early preventive treatment with Enalapril improves cardiac function and delays mortality in mice with arrhythmogenic right ventricular cardiomyopathy type 5

Short title: Preventive heart failure treatment in mice with ARVC type 5

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Abstract

Background: Arrhythmogenic right ventricular cardiomyopathy type 5 (ARVC5) is an inherited cardiac disease with complete penetrance and a very aggressive clinical course caused by mutations in *TMEM43*. There is no cure for ARVC5 and palliative treatment is started once phenotype is present. A transgenic mouse model of ARVC5 expressing human TMEM43-S358L (TMEM43mut) recapitulates the human disease, enabling the exploration of preventive treatments. The aim of this study is to determine whether preventive treatment with heart failure drugs (beta blockers (BB), angiotensin-converting enzyme inhibitors (ACEI), mineralocorticoid-receptor antagonists (MRA)) improves the disease course of ARVC5 in TMEM43mut mice.

Methods: TMEM43mut male/female mice were treated with metoprolol (BB), enalapril (ACEI), spironolactone (MRA), ACEI+MRA, ACEI+MRA+BB or left untreated. Drugs were initiated at 3 weeks of age, before ARVC5 phenotype, and serial electrocardiograms (ECG) and echocardiograms were performed.

Results: TMEM43mut mice treated with enalapril showed a significantly increased median survival compared to untreated mice (26 vs. 21 weeks; p=0.003). Enalapril treated mice also exhibited increased left ventricular ejection fraction (LVEF) at 4 months compared to controls (37.0% vs 24.9%; p=0.004), shorter QRS duration and reduced LV fibrosis. Combined regimens including enalapril also showed positive effects. Metoprolol decreased QRS voltage prematurely and resulted in a non-significant decrease in LVEF compared to untreated TMEM43mut mice.

Conclusions: Preventive enalapril-based regimens reduced fibrosis, improved ECG, echocardiographic parameters and survival of ARVC5 mice. Early metoprolol did not show positive effects and caused premature ECG abnormalities. Our findings pave the way to consider prophylactic enalapril in asymptomatic ARVC5 genetic carriers.

Word count: 250

Non-standard Abbreviations and Acronyms:

ACEI: angiotensin-converting enzyme inhibitors

ACM: Arrhythmogenic cardiomyopathy

ARCC: Arrhythmogenic right ventricular cardiomyopathy type 5 ARVC5

ARVC: Arrhythmogenic right ventricular cardiomyopathy

BB: Beta blockers

HF: Heart failure

ICD: Implantable cardiac defibrillator

LV: Left ventricle

LVEDD: Left ventricular end-diastolic diameter

LVEF: Left ventricular ejection fraction

MM: M-mode

MRA: mineralocorticoid receptor antagonists

RV: Right ventricle

SCD: Sudden cardiac death

TMEM43mut: Mice expressing TMEM43-S358L

TnI: Troponin I

Introduction

Arrhythmogenic cardiomyopathy/arrhythmogenic right ventricular cardiomyopathy (ACM/ARVC) is a rare and incurable genetically-determined disorder that causes heart failure and sudden cardiac death (SCD), predominantly in young males [1,2]. Most cases are caused by mutations in genes encoding desmosomal proteins (3-5). However, the most aggressive ACM subtype, ARVC type 5 (ARVC5) is caused by mutations in the non-desmosomal *TMEM43* gene. More specifically, the p.S358L point mutation is responsible for the majority of ARVC5 cases.

ARVC5 was first described in 2008 as a fully penetrant and sex-influenced disease with a very aggressive clinical course specially in males in families from Newfoundland (Canada) [5]. Since then, several other patients have been diagnosed in Europe and the US [6–8]. Due to the aggressive clinical course, life expectancy is severely reduced in TMEM43 genetic carriers and was reported to be as low as 41 years in males [5,9]. Current ARVC5 treatment includes SCD prevention with early implantable cardiac defibrillator (ICD) implantation and heart failure (HF) drugs treatment once systolic dysfunction appears.

We have recently reported the first characterization of a transgenic mouse model of ARVC5, which recapitulated most of the human disease [10]. Mice expressing TMEM43-S358L (TMEM43mut) present cardiomyocyte death and fibrofatty replacement and die at a young age. Furthermore, it was observed that the overexpression of the calcineurin splice variant CnAβ1 resulted in GSK3β inhibition and improved cardiac function and survival, which was also evidenced by preventive treatment with the GSK3β inhibitor SB216763 (SB2). This same agent has proved to be useful preventing myocyte injury and cardiac dysfunction in two murine models of desmosomal ACM, but its toxicity precludes its utilization in humans [11,12].

The potential benefit of preventive administration of widely available HF drugs in ARVC5 and other ACM forms is currently unknown. We sought to investigate whether early treatment with beta blockers (BB), angiotensin-converting enzyme inhibitors (ACEI), or mineralcorticoid receptor antagonists (MRA) may provide a beneficial effect in terms of cardiac function or remodeling in an ARVC5 mouse model.

Methods:

Mice

Mice (C57BL/6JCrl, Charles River Laboratories) overexpress the TMEM43 p.S358L mutation (TMEM43mut) specifically in cardiomyocytes under the control of the α-myosin heavy chain (MHC) promoter as previously described [10]. Both, male and female mice, were used in the study and were housed in an air-conditioned room with a 12-hour light/dark cycle and free access to chow and water.

Different HF drugs (metoprolol, enalapril and spironolactone) were administered dissolved in drinking water to different mice groups and were compared to a placebo group from 3 weeks of age until 4 months, when they were sacrificed. A total of 9 mice were untreated (4 male, 5 female) 11 mice were treated with metoprolol (5 male, 6 female), 13 with enalapril (6 male, 7 female), 12 with spironolactone (6 male, 6 female), 7 with a combination of enalapril plus spironolactone (2 male, 5 female) and 8 with a combination of the three drugs (triple therapy, 4 male and 4 female). The doses were 30 mg/kg for metoprolol, 20 mg/kg for enalapril and 37.5 mg/kg for spironolactone as previously reported [13,14] . Mice were sacrificed in carbon dioxide chambers at 4 months of age by gradually filling the cage. Additionally, 2 extra groups of untreated and enalapril-treated (n=17 each) were followed until mice died naturally. All experiments were approved by the CNIC Ethics Committee and the Regional Government of Madrid (PROEX-177/17).

Electrocardiograms

ECGs were obtained from anesthetized animals at 3, 5- and 10-weeks and at 4- months by using bipolar limb leads (leads I, II, and III) and unipolar limb leads (leads aVR, aVL, and aVF) for 60-90 seconds (Figure 1S). Registries were acquired by a blinded operator with mice placed under light anesthesia with isoflurane (MP36R, BIOPAC Systems, Inc.). ECGs were analyzed by an expert using Acqknowledge 4.1.1. for MP36R (BIOPAC Systems, Inc.). Mean values were calculated from 10 consecutive standard ECG time intervals and waves.

Echocardiography

Cardiac function, chamber dilatation, and wall thickness were analyzed in TMEM43mut mice aged 3 weeks, 5 weeks and 4 months by transthoracic two-dimensional (2D), and M-mode (MM) echocardiography (Figure 1S). A blinded operator carried out measurements using a high-frequency ultrasound system with a 40-MHz linear probe (Vevo 2100, Visualsonics Inc., Canada). During ultrasound scans, mice were kept under light anesthesia with 0.5-2% isoflurane in oxygen adjusted to obtain a target heart rate of 500±50 bpm and were placed on a heating pad at 38.3^{o}C. Left ventricular (LV) ejection fraction (LVEF) and volumes were obtained from the 2D long axis view. LV diameters were obtained with M-mode from the short axis view. Right ventricular (RV) systolic function was assessed indirectly from the tricuspid annular plane systolic excursion (TAPSE), estimated from maximum lateral tricuspid annulus movement obtained from a 2D 4-chamber apical view using M-mode. Images were analyzed off-line by a blinded expert using the Vevo 2100 Workstation software.

Troponin determinations

Serum troponin I (TnI, ng/ml) was measured by ELISA in mice aged 3, 5, 10 weeks, and 4 months as previously described [10].

Histological analysis

Mice, hearts and lungs were weighed after sacrifice. Heart sections were analyzed using Masson's trichrome and picrosirius red (PSR) staining and viewed using a Nikon 90i microscope (bright field and polarized light). Images were processed with NIS Elements 3.2 software.

Statistical analysis

Data are presented as means ±SEM. All datasets were analyzed for statistical significance by regular or repeated measures one-way ANOVA followed by Bonferroni's for multiple comparisons, or two-way ANOVA followed by Bonferroni's

post-test (GraphPad Prism and SPSS v20). Survival curves were compared by the log-rank (Mantel-Cox) test. Differences were considered statistically significant at $p<0.05$.

The authors of this manuscript make the data, methods used in the analysis and materials used to conduct the research available to any researcher for purposes of reproducing the results or replicating the procedure

Results:

Enalapril reduces electrophysiological alterations in ARVC5 mice

A total of 60 TMEM43mut mice (27 male, 33 female) were treated with HF drugs already approved for human use, including metoprolol, enalapril, spironolactone, a combination of enalapril plus spironolactone and a combination of the three drugs. Mice were followed for 4 months and compared to controls. All treatment drugs were well tolerated by mice and no adverse events were observed. Three mice included in these treatment groups died before the 4 months: one in the metoprolol group at 8 weeks, one in the untreated group at 6 weeks and another one in the triple therapy group at 14 weeks.

Blood pressure and heart rate were measured in conscious mice at 10 weeks for all treatment groups. Metoprolol treated mice had a significantly lower mean heart rate (641 ± 17 bpm vs. 681 ± 6 bpm in mice without metoprolol; p=0.009) , whereas enalapril and triple therapy treated mice showed lower mean blood pressure (57 ± 5 mmHg and 54 ± 3 mmHg vs. 70 ± 3 mmHg in the untreated group; p=0.045 and 0.025, respectively) (Figure 2S).

Untreated mice showed a progressive increase in P wave duration, which was statistically significant from week 5 to week 10 (8.3 ± 0.3 ms vs. 12.5 ± 0.3 mmHg; $p<0.001$). These same findings were observed in metoprolol treated mice, but not in the other groups. At 10 weeks and 4 months, all mice treated with enalapril, alone or in combination with other drugs, presented significantly narrower P waves compared to untreated mice (Figure 1A). Similarly, QRS duration was significantly shorter in TMEM43mut mice treated with enalapril-based regimens at 4 months (10.4 ± 0.4 ms in enalapril treated mice vs. 13.6 ± 0.6 ms in untreated; $p<0.001$). (Figure 1B). Enalapril

and enalapril + spironolactone treated mice were the only groups that did not significantly increase QRS duration over time (3 weeks vs. 4 months). QRS voltage decreased similarly with time in all groups (Figure 2A). However, mice treated with metoprolol presented a premature decrease in QRS voltage by week 10, which showed a statistical trend compared to untreated mice at this stage (0.4 ± 0.04 mv vs. 0.8 ± 0.04 ng/ml; p=0.06) (Figure 2A, 2B). No ventricular arrhythmias or significant conduction abnormalities were observed in the ECG at the different time points neither in metoprolol nor in enalapril treated groups (Figure 2B).

Enalapril improves systolic dysfunction in ARVC5 mice

At 4 months of age, all groups presented a significantly decreased left ventricular ejection fraction (LVEF) compared to 3 weeks of age (Table 1). Metoprolol treated mice showed further decrease in LVEF at 4 months compared with the control group, although differences were not significant (Table 1). Mice treated with enalapril or enalapril + spironolactone showed significantly improved LVEF at 4 months of age compared to the untreated and the metoprolol groups (37.0 ± 1.0% and 39.9 ± 0.5% vs. 24.9± 3.0% and 21.1 ± 3.2%, respectively; $p<0.05$ for all comparisons). (Figure 3A). Regarding mice treated with triple therapy, no differences were observed compared to untreated and metoprolol groups at 4 months.

Left ventricular end-diastolic diameter (LVEDD) increased with age in all groups (Table 2). Untreated and metoprolol treated mice presented the largest LVEDD at 4 months of age, although differences were not significant compared to the other groups with enalapril-based regimens (Figure 3B).

All groups, with the exception of the triple therapy and enalapril + spironolactone groups, presented a significant decrease of right ventricular systolic function assessed by TAPSE (Table 1). By 4 months of age, only mice with triple therapy presented a significantly higher TAPSE compared to the untreated group.

No significant differences in ECG or ehocardiography were observed between males and females in the different treatment groups.

Enalapril decreases cardiomyocyte death in mice expressing the mutant TMEM43 protein

We have previously shown that expression of human TMEM43.pS358L leads to cardiomyocyte death and increased circulating cardiac troponin (cTnI) in ARVC5 mice [10]. To determine the effect of the drugs on cardiomyocyte death, circulating cTnI was analyzed at different time points in the enalapril, metoprolol and untreated groups of TMEM43mut mice. We found that enalapril delayed the increase of cTnI, with significantly lower values than the control group observed at 10 weeks (1.8 ± 0.3 ng/ml vs. 0.7 ± 0.1 ng/ml; p=0.013). (Figure 4A). This reduced cTnI was paralleled by a significantly improved LVEF at this time point compared to untreated mice (Figure 4B). Additionally, enalapril treated mice showed lower levels of the heart failure markers BNP (Nppb) and Acta 1 in myocardial tissue at 10 weeks and 4 months compared to untreated mice, although it only reached statistical significance at 4 months (Figure 4C and 4D). Together, these results suggest that enalapril delays cardiomyocyte death and the overall progression of the disease.

Enalapril reduces cardiac fibrosis in ARVC5 mice

Cardiomyocyte death induced by TMEM43-S358L progressively leads to replacement fibrosis [10]. To determine whether the reduction in cardiomyocyte death in the presence of enalapril, indicated by the decreased presence of cTnI in serum, would lead to a reduction in myocardial fibrosis, fibrotic tissue was examined in explanted hearts at 10 weeks and 4 months in untreated, metoprolol and enalapril treated mice. Masson's trichrome staining revealed a significantly reduced fibrotic area in the enalapril group both at 10 weeks and 4 months (Figure 5A, 5B). Hearts of mice treated with metoprolol presented a similar amount of fibrosis compared to untreated mice. PSR staining did not reveal significant differences in the type of collagen in the three groups. The percentage of type I collagen, which confers stiffness to the tissue, significantly increased in all groups, accounting for 70-80% of total collagen at 4 months. (Figure 5C).

Enalapril improves survival of ARVC5 mice

To investigate whether the reduced cardiomyocyte death and fibrosis that results from treatment with enalapril would improve survival of ARVC5 mice, two additional groups of 17 untreated and 17 enalapril-treated animals were followed until mice died naturally. Enalapril treated mice presented a significantly longer median survival (26 weeks vs 21 weeks in the untreated group; p=0.003) (Figure 5D). Metoprolol and spironolactone groups were not evaluated for survival due to lack of significant functional improvement at 4 months of age, when initial animal groups were sacrificed by protocol.

Discussion

ARVC5 is an aggressive genetic cardiomyopathy for which there is currently no effective pharmacologic treatment available. We have previously shown that a transgenic mouse model of ARVC5 expressing the human TMEM43-S358L protein develops progressive biventricular systolic dysfunction and accumulation of fibro-fatty tissue, reproducing the human disease [10]. In this study we used this model to explore the potential benefit of drugs already approved for human heart failure for the preventive treatment of ARVC. Since the first disease manifestations in these mice are electrical abnormalities with a decrease in QRS voltages already seen at 5 weeks of age [10], we started preventive therapies at 3 weeks of age with the aim to delay the onset of electrical and structural abnormalities. We found that although none of the studied drugs was able to prevent completely the development of ARVC, enalapril-based regimens delayed the onset of disease, preserved cardiac function and improved survival compared to untreated mice.

To date, heart failure medical therapy in patients with ACM/ARVC and LV dysfunction follows the current ESC and ACC/AHA/HFSA heart failure guidelines recommendations for dilated cardiomyopathy (DCM) [15,16]. BB and ACEI are recommended when LVEF is below 40% and MRA is added if patients remain symptomatic and LVEF is below 35% [15,16]. Angiotensin receptor–neprilysin inhibitor (ARNI) valsartan/sacubitril and sinoatrial node modulators (ivabradine) are included if no response is seen with BB, ACEI and

MRA. For RV dysfunction, there is even less clinical evidence and currently ACEI, BB and MRA are only recommended when RV systolic dysfunction is already present [17]. However, none of these drugs are recommended as preventive agents. Preload reducers (diuretics and isosorbide nitrate) prevented the development of ARVC in a plakoglobin knockdown mouse [18], suggesting that these agents may be useful in early stages of desmosomal ARVC [17]. However, to date preventive treatments are not routinely used in ARVC5 mutation carriers or ACM/ARVC mutation carriers in general. The aim of early initiation of medical therapy in these patients would be to prevent arrhythmic complications and also to control ventricular dimensions and function [17].

Here, we observed that treatment of ARVC5 mice with enalapril-based regimens started at week 3, when mice still do not show any pathological features, had a positive impact on ECG parameters by week 10, including a narrower P wave. Furthermore, enalapril treated mice prevented the QRS widening observed in untreated mice. These results are concordant with the milder reduction in LVEF observed at 4 months in the enalapril-treated groups compared to untreated mice, which showed a strong decline in LV contractility. Moreover, TMEM43mut mice treated with triple therapy also had improved RV function as assessed by higher TAPSE at 4 months compared to untreated mice. The preservation of cardiac function promoted by enalapril resulted in an increased lifespan, with a 5 weeks longer median survival compared to untreated mice. Mean arterial blood pressure was also significantly lower in enalapril treated mice (Figure 2S), which could partly explain its benefit in TMEM43mut mice due to the cardioprotective effect of lowering blood pressure.

In humans, ACE inhibitors have proven to decrease mortality in both symptomatic and asymptomatic patients with left ventricular dysfunction [19]. There are currently no recommendations for treatment of DCM or ARVC/ACM mutation carriers with normal LVEF, but there is an ongoing trial with HF drugs including ACE inhibitors in patients with Duchenne muscular dystrophy without DCM [20] in order to try to prevent cardiomyopathy onset. Our results suggest for the first time that early initiation of enalapril is useful to halt progression of arrhythmogenic cardiomyopathy phenotype

and pave the way for future studies investigating prophylactic enalapril treatment in asymptomatic ARVC genetic carriers.

In contrast, prophylactic treatment with metoprolol did not produce a beneficial effect. Indeed, it seemed it could be deleterious as metoprolol-treated mice showed a statistical trend towards a decreased QRS voltage compared to untreated mice. Moreover, metoprolol treatment also resulted in the largest LV diameters compared to enalapril-based regimens. This apparent deleterious effect of preventive treatment with BB is not applicable to other murine cardiomyopathy models. For instance, in a model bearing a TNNT2 deletion oral metoprolol prevented cardiac dysfunction and extended survival compared to other BB and placebo [13]. Extension of the results observed with metoprolol in our ARVC5 model to humans is not straightforward. In humans, DCM caused by mutations in other nuclear envelope genes like *LMNA* and *EMD* have an increased risk for conduction disorders, so BB should be used with caution in subjects harboring genetic variants in these genes. However, ARVC5 patients do not show this conduction disease phenotype and there are no specific recommendations regarding BB use. The potential usefulness of BB to treat ARVC is based on their efficacy to prevent SCD and heart failure progression; however, there are no randomized clinical trials comparing BB with placebo in ARVC/ACM [21].

We have previously shown that TMEM43-S358L induces cardiomyocyte death, which results in the release of cTnI to the bloodstream and in the replacement of dead cardiomyocytes by fibrotic tissue [10]. Enalapril treated mice presented reduced fibrosis at 10 weeks and 4 months compared to other groups. Additionally, heart failure markers BNP and Acta 1 were significantly decreased compared to untreated mice at 4 months. These findings correlated with the observed delayed peak in cTnI, suggesting that the mechanism by which enalapril improved cardiac function in ARVC5 mice involved, at least in part, a delay in cardiomyocyte death and a slower progression of the disease [22].

ACE inhibitors reduce myocardial fibrosis and improve ventricular remodeling in different murine models including Duchenne's related DCM, myocarditis and myocardial infarction [14,23,24]. Specifically, enalapril has been shown to attenuate apoptosis, reduce inflammation and improve cardiac function after myocardial infarction in mice [25], [26]. However, there are no data about its effects in ARVC/ACM. Inflammation has recently emerged as a potential mechanism in the pathogenesis of ARVC/ACM [27] and periodic bursts or "hot phases" with arrhythmia and cTnI elevations have been described in disease progression [28]. Moreover, autopsy studies have shown inflammatory infiltrates in patients with this disease [29], suggesting that the anti-inflammatory properties of ACE inhibitors [30] could be of particular benefit in ARVC/ACM.

To our knowledge, this is the first study to show a beneficial effect of enalapril on ARVC/ACM. The fact that it improves cardiac function, it delays the onset of the disease and it improves survival in a mouse model of ARVC5, one of the most aggressive subtypes of ARVC/ACM, provides grounds for optimism and opens the way for studies in human ARVC/ACM.

Limitations

All treatments were administered in drinking water, so the exact doses received by each mouse could slightly vary depending on water intake. On the other hand, this route of administration is non-invasive and more physiological and drug intake was confirmed by reduced heart rate and the lower blood pressure observed in the active medication groups. Survival analysis was only performed in untreated and enalapril treated TMEM43mut mice; this information is lacking in the other experimental groups, which were sacrificed at 4 months of age. Therefore, conclusions on drug efficacy in these groups are only based on ECGs, echocardiograms and histological features and not on survival outcomes.

Conclusions:

Enalapril-based regimens improve ECG and echocardiogram features as well as survival of ARVC5 mice when therapy is initiated before the first pathological manifestations have appeared. On the other hand, preventive treatment with metoprolol failed to show positive effects and could be deleterious as reflected by premature ECG anomalies. Future studies will determine whether early initiation of enalapril can delay the disease onset in human ARVC5 genetic carriers.

What is new?

- This is the first study to evaluate the effect of preventive heart failure treatment in an animal model of ARVC5.
- Treatment with Enalapril delayed the onset of the disease in ARVC5 mice and improved survival. In contrast, preventive treatment with metoprolol had a negative impact on ARVC5 causing premature ECG abnormalities.

What are the clinical implications?

- ARVC5 is the most aggressive form of arrhythmogenic cardiomyopathy and there are currently no effective therapies to prevent its progression in humans.
- Enalapril is a well-tolerated, affordable and widely used drug that could have a role as a preventive therapy for ARVC5 in humans. Observational studies with asymptomatic ARVC5 genetic carriers will determine its usefulness in humans

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Table 1

Group	Parameter	3 weeks	5 weeks	4 months
Untreated (n= 9)	LVEF (% ± SEM)	65.0 ± 3.0	59.5 ± 2.5	24.9 ± 2.6**
	TAPSE (mm±SEM)	1.0 ± 0.2	1.1 ± 0.2	0.7 ± 0.2*
Metoprolol (n=11)	LVEF (%± SEM)	62.2 ± 0.9	56.0 ± 2.3	21.1 ± 3.2**
	TAPSE (mm±SEM)	1.0 ± 0.2	1.1 ± 0.2	0.6 ± 0.1**
Spironolactone (n=12)	LVEF (%± SEM)	67.2 ± 1.3	61.4 ± 1.0	30.1 ± 1.8**
	TAPSE (mm±SEM)	1.0 ± 0.1	1.0 ± 0.2	0.7 ± 0.1**
Enalapril (n=13)	LVEF (%± SEM)	64.3 ± 1.6	61.0 ± 1.1	37.0 ± 1.0**,+
	TAPSE (mm±SEM)	1.1 ± 0.1	0.9 ± 0.1	0.8 ± 0.1*
Triple therapy (n=8)	LVEF (%± SEM)	66.3 ± 1.1	59.6 ± 1.4	33.8 ± 1.7**
	TAPSE (mm±SEM)	1.1 ± 0.2	1.0 ± 0.1	0.9 ± 0.1+
Enalapril + spironolactone (n=7)	LVEF (%± SEM)	62.8 ± 1.6	61.4 ± 1.0	39.9 ± 0.7**,+
	TAPSE (mm±SEM)	1.1 ± 0.1	1.1 ± 0.1	0.8 ± 0.1

Table 1. Left and right ventricular systolic function over time in different treatment groups. LVEF: Left ventricular ejection fraction (%), TAPSE: Tricuspid annular plane systolic excursion (mm). SEM: Standard error mean

*p<0.05 4 months vs. 3 weeks

**p<0.01 4 months vs 3 weeks

+p<0.05 vs. untreated group 4 months, Two-way ANOVA followed by Bonferroni post-test.

Table 2

Group	Parameter	3 weeks	5 weeks	4 months
Untreated (n=9)	LVAWD (mm±SEM)	0.71 ± 0.01	0.78 ± 0.04	0.79 ± 0.05
	LVPWD (mm±SEM)	0.79 ± 0.14	0.70 ± 0.08	0.71 ± 0.04
	LVEDD (mm±SEM)	2.97± 0.18	3.27 ± 0.12	4.44 ± 0.20*
	HW/BW (±SEM)	-	-	6.3 ± 0.8
Metoprolol (n=11)	LVAWD (mm±SEM)	0.61 ± 0.03	0.73 ± 0.03	0.72 ± 0.03
	LVPWD (mm±SEM)	0.70 ± 0.03	0.74 ± 0.06	0.66 ± 0.03
	LVEDD (mm±SEM)	3.21± 0.10	3.56 ± 0.15	4.59 ± 0.08*
	HW/BW (±SEM)	-	-	7.8 ± 0.5
Spironolactone (n=12)	LVAWD (mm±SEM)	0.70 ± 0.03	0.70 ± 0.02	0.76 ± 0.03
	LVPWD (mm±SEM)	0.59 ± 0.06	0.68 ± 0.04	0.65 ± 0.03
	LVEDD (mm±SEM)	3.20± 0.07	3.34 ± 0.09	4.34 ± 0.07*
	HW/BW	-	-	6.9 ± 0.5
Enalapril (n=13)	LVAWD (mm±SEM)	0.73 ± 0.02	0.76 ± 0.03	0.68 ± 0.02
	LVPWD (mm±SEM)	0.69 ± 0.03	0.67 ± 0.04	0.68 ± 0.02
	LVEDD (mm±SEM)	3.06 ± 0.09	3.46 ± 0.08	4.21 ± 0.05*,#
	HW/BW(±SEM)	-	-	5.3 ± 0.3+
Triple therapy (n=8)	LVAWD (mm±SEM)	0.77 ± 0.04	0.73 ± 0.02	0.72 ± 0.05
	LVPWD (mm±SEM)	0.63 ± 0.02	0.60 ± 0.03	0.77 ± 0.07
	LVEDD (mm±SEM)	3.19 ± 0.06	3.84 ± 0.06	4.29 ± 0.10*
	HW/BW(±SEM)	-	-	5.0 ± 0.3+

Enalapril + spironolactone (n=7)	LVAWD (mm±SEM)	0.68 ± 0.04	0.60 ± 0.05	0.68 ± 0.02
	LVPWD (mm±SEM)	0.70 ± 0.05	0.65 ± 0.05	0.60 ± 0.03
	LVEDD (mm±SEM)	3.45 ± 0.07	3.80 ± 0.15	4.33 ± 0.09*
	HW/BW(±SEM)	-	-	4.8 ± 0.2^{+}

Table 2. Left ventricular wall widths, diastolic dimensions over time and heart weight/body weight (HW/BW) at 4 months in different treatment groups.
LVAWd: : LV anterior wall thickness in diastole
LVPWd: LV posterior wall thickness in diastole
LVEDD: LV end diastolic diameter
SEM: Standard error mean
*p<0.01 4 months vs 3 weeks
$^{\#}$p=0.07 enalapril 4 months vs metoprolol 4 months
$^{+}$p<0.05 vs metoprolol

FIGURE LEGENDS

Figure 1. Enalapril reduces P wave and QRS duration in ARVC5 mice. **A, B**, Mice expressing human TMEM43-S358L in cardiomyocytes were treated with the indicated HF drugs starting at 3 weeks of age and P waves (A) and QRS complexes (B) duration were measured by ECG at 3, 5 and 10 weeks and at 4 months. Boxes show the interquartile range (Q1-Q3). The whiskers at the end of each vertical line show minimum and maximum values. Median is represented by the horizontal bar in each box and mean is represented as "+". *p <0.05 each group vs. untreated mice, 2-way ANOVA and Bonferroni post-test. n=8-13.

Figure 2. Effect of HF medications in QRS amplitude. **A**, ARVC5 mice were treated with different HF drugs and QRS amplitude in the ECG was measured at 3, 5 and 10 weeks and at 4 months. Boxes show the interquartile range (Q1-Q3). The whiskers at the end of each vertical line show minimum and maximum values. Median is represented by the horizontal bar in each box and mean is represented as "+". **B**, Representative ECG tracings of untreated mice and mice treated with metoprolol and enalapril are shown at different time points. W, weeks; M, months. *p=0.06 Metoprolol vs. untreated mice. 2-way ANOVA and Bonferroni post-test. n=8-13.

Figure 3. Enalapril improves cardiac function in ARVC5 mice. **A, B**, ARVC5 mice were treated with different HF drugs starting at 3 weeks and left ventricular end diastolic diameter (LVEDD; A) and left ventricular ejection fraction (LVEF; B) were measured by echocardiography at 3 and 5 weeks and at 4 months of age. Boxes show the interquartile range (Q1-Q3). The whiskers at the end of each vertical line show minimum and maximum values. Median is represented by the vertical bar in each box and mean is represented as "+". *p<0.05 treated with each drug vs. untreated at 4 months. Two-way ANOVA plus Bonferroni post-test. n=8-13.

Figure 4. Enalapril delays cardiomyocyte death and systolic dysfunction in mice expressing TMEM43-S358L. A, Circulating cardiac Troponin I was measured by ELISA in untreated, metoprolol and enalapril groups at 3, 5, 10 weeks and at 4, 5 months. **B,** LVEF was determined by echocardiography in untreated, metoprolol and enalapril treated mice at 3, 5, 10 weeks and at 4 months. Boxes show the interquartile range (Q1-Q3). The whiskers at the end of each vertical line show minimum and maximum values. Median is represented by the horizontal bar in each box and mean is represented as "+". **C, D**, Acta1 (C) and BNP (D) mRNA expression was determined by qRT-PCR in heart tissue at 10 weeks and 4 months.. *p <0.05, Enalapril vs. untreated; +p<0.05, metoprolol 5 weeks vs. 3 weeks; $^{\#}$p<0.05, all groups vs 3 weeks, $^{++}$p<0.01

enalapril vs. untreated and metoprolol treated mice. $p=0.11 vs.untreated. **p<0.05 vs. untreated Two-way ANOVA plus Bonferroni post-test. n=8-10.

Figure 5. **Enalapril reduces fibrosis and improves survival in ARVC5 mice**. **A,** Mice expressing TMEM43-S358L were treated with enalapril, metoprolol or were left untreated from 3 weeks of age and were analyzed at 4 months. Representative images of explanted hearts, Masson's trichrome staining showing areas of fibrosis (pink) and polarized light images with picrosirius red (PSR) staining are shown. Scale, 50 μm. **B,** Ratio of fibrosis/total tissue in untreated mice and mice treated with metoprolol or enalapril. **C,** The collagen type in the different treatment groups was quantified from polarized light images and represented as percentage of collagens with increasing stiffness. Collagen type I (red) is stiffer and collagen type III (green) more elastic. Intermediate collagens are represented in orange and yellow. **D,** Survival curve comparing untreated mice (n=17) with Enalapril-treated mice (n=17). *p<0.05 HF drug vs Untreated, +p <0.001 4 months vs 10 weeks, #p <0.05 4 months vs 10 weeks, $p<0.001 Col III & Col I 4 months vs 10 weeks
Two-way ANOVA for B and C. n=8-10.

Supplemental Materials:

Figure 1S. Study protocol

Figure 2S. Mean arterial blood pressure (mmHg) and mean heart rate (beats per minute). All mice were monitored at week 10. Boxes show the interquartile range (Q1-Q3). The whiskers at the end of each vertical line show minimum and maximum values. Median is represented by the vertical bar in each box and mean is represented as "+".

*p<0.05 vs. Untreated; #p<0.01. Metoprolol-treated mice vs. all untreated mice in the other groups.