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## Mouse models of spontaneous atrial fibrillation

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### Abstract

Atrial fibrillation (AF) is the most common arrhythmia in adults, with a prevalence increasing with age. Current clinical management of AF is focused on tertiary prevention (i.e., treating the symptoms and sequelae) rather than addressing the underlying molecular pathophysiology. Robust animal models of AF, particularly those that do not require supraphysiologic stimuli to induce AF (i.e., showing spontaneous AF), enable studies that can uncover the underlying mechanisms of AF. Several mouse models of AF have been described to exhibit spontaneous AF, but pathophysiologic drivers of AF differ among models. Here, we describe relevant AF mechanisms and provide an overview of large and small animal models of AF. We then provide an in-depth review of the spontaneous mouse models of AF, highlighting the relevant AF mechanisms for each model.

### Introduction

AF affects 1–2% of adults > 20 years old in the United States as well as an estimated 46.3 million individuals worldwide (Benjamin et al. 2019), with 70% of individuals with AF being older than 65 years old (Feinberg et al. 1995). Data from the Framingham Heart Study have demonstrated a three-fold increase in AF prevalence over the last 50 years (Schnabel et al. 2015). AF is a progressive arrhythmia disorder (Wijffels et al. 1995), with AF risk increasing with age. Patients typically progress from paroxysmal AF, which spontaneously terminates within seven days, to persistent AF to long-standing persistent AF, which lasts more than 12 months. Treatment of acute AF involves rate control with

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beta blockers, non-dihydropyridine calcium channel blockers, or digoxin for patients with decompensated heart failure (HF). Patients who fail rate control are treated with rhythm control via pharmacologic, electrical, or interventional cardioversion. Anticoagulation is given to patients as guided by the CHA<sub>2</sub>DS<sub>2</sub>-VASc score. In addition to having adverse side effects, these therapies are limited in that they only address the symptoms and sequelae of AF, not the underlying cause. Moreover, while anticoagulation decreases risk of stroke up to 60% (Piccini and Fonarow 2016), patients still have a residual stroke risk along with greater risk of HF, cognitive impairment, and mortality. Thus, better treatments targeting the underlying pathobiology of AF are needed for the primary and secondary prevention of AF. Robust animal models are critical in understanding such molecular mechanisms as well as uncovering novel therapeutic targets.

## Atrial fibrillation mechanisms

In this review, key aspects of AF mechanisms are described with a focus on those recapitulated in animal models. The two major mechanisms contributing to the formation of AF are reentry and ectopic electrical activity (Fig. 1) (Heijman et al. 2016). Reentry-prone substrates are formed because of electrical and structural remodeling. Structural remodeling is characterized by left atrial (LA) enlargement and fibrosis, which creates heterogenous conduction and alters cardiomyocyte excitability (Fig. 1). Electrical remodeling is characterized by abbreviated refractoriness and slowed conduction (Nattel and Dobrev 2016). Ectopic electrical activity, which provides the actual trigger to initiate arrhythmic episodes, occurs in the form of abnormal automaticity, early after-depolarizations (EADs), or delayed after-depolarization (DADs), all of which can trigger an ectopic action potential (Fig. 1) (Wit and Boyden 2007).

Abnormal automaticity often arises in ectopic pacemaker cells and is due to upregulation of the hyperpolarization-activated cyclic nucleotide-gated (HCN) funny current (Tse 2016). EADs occur when the refractory period is prolonged due to premature reactivation of the sarcolemmal voltage-gated L-type calcium channel (LTCC) prior to full repolarization. In some cases, EADs occur due to premature reactivation of fast sodium currents (Sato et al. 2017). However, DADs are thought to be the predominant AF trigger and arise in states of abbreviated refractoriness such as secondary to spontaneous ryanodine receptor (RyR2)-mediated sarcoplasmic reticulum (SR) diastolic calcium leak (Wit and Boyden 2007). Spontaneous diastolic calcium release events can activate the sodium/calcium exchanger (NCX)—which is upregulated in patients with persistent AF (Voigt et al. 2012)—to pump out one calcium ion in exchange for three sodium ions, resulting in a net depolarizing current that can trigger an action potential (Voigt et al. 2012). Repeated focal ectopic firing can also sustain AF in the absence of an AF-prone substrate or initiate an AF-sustaining reentrant circuit.

One important mechanistic driver of reentry and ectopic electrical activity is oxidative stress. Oxidative stress has been shown to result in atrial electrical remodeling, characterized by increased SR calcium leak (Joseph et al. 2016), conduction slowing via alterations in gap junction expression (Gemel et al. 2017; Smyth et al. 2010), and decreased inward sodium current density (Liu et al. 2012). Oxidative stress also causes atrial structural remodeling

through several pathways such as the receptor for advanced glycation endproducts (RAGE) axis (Kato et al. 2008), transforming growth factor (TGF)  $\beta$ 1 expression (Mira et al. 2013), and the Ras homolog gene family member A (RhoA) GTPase/ROCK pathway (Chen et al. 2014; Sah et al. 1999). Oxidative stress is a key determinant of AF susceptibility and contributes to spontaneous AF in many of the mouse models discussed below.

## Overview of animal models of atrial fibrillation

### Large animal models

Large animals of AF include goats, dogs, sheep, pigs, and horses. As discussed (Frydrychowski et al. 2020), advantages of large animal models include the possibility of detailed experimental procedures, in-depth determination of cardiac function, high availability of tissue samples (Odening et al. 2021) and anatomical-physiological similarity to human hearts. However, these models often incur higher experimental costs and longer study periods. Moreover, spontaneous AF is uncommon except in dogs (Buchanan 1965) and horses (Hesselkilde et al. 2019). In large animal models, AF is often induced via atrial structural remodeling or rate-induced electrical remodeling such as atrial burst pacing to shorten ERP (Nishida et al. 2010). AF in goats (Doddall et al. 2013), sheep (Anne et al. 2007), and dogs (Cardin et al. 2007) is induced via atrial tachypacing (ATP) and reflects the progressive nature of human AF as maintenance of AF progressively shortens the atrial ERP and increases AF duration. Sheep enable the study of ischemia-induced AF due to the presence of a unique coronary branch supplying the posterior LA (Avula et al. 2018). Spontaneous AF in dogs is associated with structural heart disease (e.g., myxomatous mitral valve disease (Jung et al. 2016)) and carries mechanistic similarities to humans as canine pulmonary veins have a shorter action potential duration (APD) compared to the surrounding LA (Ehrlich et al. 2003). While similarity to humans in terms of size and EP properties as well as ability for genetic manipulation (e.g., Brugada syndrome (Park et al. 2015)) make pigs an ideal model, pigs are prone to systolic HF after ATP-induced AF (Lugenbiel et al. 2015). Horses have been reported to exhibit spontaneous AF (Hesselkilde et al. 2019) and have human-like structural and electrical remodeling with AF-associated ectopy originating in the PVs.

### Small animal models

Small animal models include mice, rats, guinea pigs, and rabbits. These models are ideal for hypothesis generation, but the requirement for significant physiologic perturbations to induce AF may limit their translatability (Fu et al. 2022). Nonetheless, small animal models are advantageous over large animal models in terms easy generation, lower cost, and possibility of genetic manipulation (Dobrev and Wehrens 2018). Guinea pigs and rabbits are advantageous over mouse models as the presence of a calcium-driven plateau phase during the action potential makes them suitable models for studying repolarization (Clauss et al. 2019). Moreover, the relatively larger atrial size makes experimental manipulation easier. Disadvantages of guinea pigs and rabbits include increased costs, more difficult genetic manipulation, and short AF duration on the order of seconds (Suzuki et al. 2014).

Mice are the most widely used animal model of AF. Most murine AF models require generation of an AF-predisposing substrate (e.g., TGF- $\beta$ 1 overexpression) (Verheule et al. 2004). Advantages of mouse models include wide accessibility, affordable genetic manipulation, genetic homology with humans, and human-like phenotypic effects of monogenic conditions, i.e., monogenic channelopathies. Disadvantages of mouse models include a high resting heart rate, different ion channel expression and APD compared to humans, limited EP instrumentation (e.g., catheters), and low intracardiac mapping resolution. Here, we discuss four murine models of spontaneous AF in detail, as they recapitulate the pathophysiology of human AF by developing a vulnerable substrate and progressing to spontaneously induced AF episodes.

## Mouse models of spontaneous AF

Spontaneous AF, or AF not requiring an exogenous electrical stimulus, has been reported in several mouse models (Schuttler et al. 2020). Here, we describe all spontaneous AF mouse models to our knowledge and present in greater detail the four main spontaneous AF models that have been more extensively characterized—liver-kinase B1 (LKB1) knockout (KO), cyclic AMP-responsive element modulator (CREm)-transgenic (Tg) overexpression, angiotensin-converting enzyme (ACE)-Tg overexpression, and tumor necrosis factor alpha (TNF- $\alpha$ ) Tg overexpression. In Table 1, we summarize the relevant pathophysiologic features present in each spontaneous AF model (i.e., fibrosis, changes in ERP, slowed conduction, DADs, EADs, and calcium mishandling).

### **Lkb1-KO**

*LKB1* is a tumor suppressor, originally discovered as the causal loss-of-function mutation in Peutz-Jeghers syndrome (Hemminki et al. 1998), a premalignant syndrome characterized by gastrointestinal (GI) hamartomatous polyps and predisposition to solid organ cancers in the GI tract, breast, testis, and ovary (Giardiello et al. 1987). AMP-activated kinases (AMPKs) are important downstream mediators of LKB1 signaling (Shackelford and Shaw 2009). Loss of *LKB1* in most tissues is characterized by hyperplasia while loss of *LKB1* in the heart results in hypertrophy due to the non-dividing nature of cardiomyocytes (Ikeda et al. 2009). To this end, cardiomyocyte-specific *Lkb1*-KO (*Lkb1*-CKO) mice were generated by crossing the alpha-myosin heavy chain ( $\alpha$ MHC)-Cre allele with the *Lkb1*-floxed allele (Ikeda et al. 2009). *Lkb1*-CKO mice developed bi-atrial enlargement with 100% AF incidence at five weeks of age. In *Lkb1*-CKO mice, collagen expression was increased three-fold by four weeks of age and histological evidence of atrial fibrosis was apparent at 12 weeks of age, followed by left ventricular (LV) hypertrophy. Ozcan et al. (2015) built upon the study by Ikeda et al. (2009) by performing a more detailed characterization of the arrhythmic phenotype observed in *Lkb1*-CKO mice. The prevalence of spontaneous AF increased with age, from 47% at four weeks-of-age to 74% at six weeks and 95% at 12 weeks (Fig. 2A). In *Lkb1*-CKO mice, arrhythmia progression began with paroxysmal AF without changes in baseline heart rate (Ozcan et al. 2015). Mice then became more bradycardic as they progressed into persistent AF. PR, QRS, and QT intervals were unchanged while *Lkb1*-CKO mice were in sinus rhythm, but PR and QRS intervals increased at the onset of paroxysmal AF. Mice in persistent AF also gained weight and showed concomitant signs of HF (Ozcan

et al. 2015). While LV systolic function was preserved in mice with sinus rhythm and paroxysmal AF, LV ejection fraction (EF) decreased in mice with persistent AF. Despite these ventricular changes, however, an early morphologic hallmark of loss of cardiac *Lkb1* was bi-atrial dilation, beginning prior to the onset of paroxysmal AF. Biatrial dilation was accompanied by fibrosis and infiltration of pro-inflammatory immune cells including macrophages, neutrophils, and lymphocytes. Strikingly, these morphologic changes were observed in *Lkb1*-CKO mice while they were still in sinus rhythm; fibrosis and immune cell infiltration increased as mice progressed into paroxysmal and persistent AF (Ozcan et al. 2015). Beginning prior to the onset of paroxysmal AF onset, *Lkb1*-CKO mice also demonstrated signs of decreased exercise capacity, which became progressively worse as mice progressed into paroxysmal and persistent AF. Survival in *Lkb1* KO mice was 94% at six weeks, 82% at three months, and 65% at six months; common causes of death included HF and sudden cardiac death. In mice with AF, ventricular fibrillation (VF) was the most common cause of death (Ozcan et al. 2015). Interestingly, 18.8% of *Lkb1* KO female mice died during pregnancy secondary to peripartum cardiomyopathy. *Lkb1* KO mice also had decreased phosphorylated AMPK with concomitant evidence of atrial metabolic and oxidative stress. Indeed, a recent study demonstrated that administration of AMPK activators metformin and aspirin attenuated AF incidence by eightfold in *Lkb1* KO mice with concomitant improvement in mitochondrial function, gap junction protein expression, and attenuated fibrosis (Ozcan et al. 2021). Altogether, *Lkb1* KO mice have a characteristic human-like arrhythmia progression from sinus rhythm into paroxysmal and persistent AF with concomitant electrical and structural remodeling. Moreover, like in humans, premature atrial complexes (PACs), atrioventricular (AV) block, and atrial flutter predicted risk of progression into AF. Thus, loss of *Lkb1* in the heart is a robust and translatable murine model of AF with mechanistic findings like human AF.

Recently, Fu et al. (2022) studied the AF phenotype of the cardiomyocyte-specific *Lkb1*-CKO mouse in detail and reported that the electrocardiographic findings were representative of human AF (i.e., irregularly irregular R-R intervals without discernable P waves) (Fu et al. 2022). However, this study found that the electrocardiographic phenotype could be secondary to severe atrial cardiomyopathy, which prevented the conduction of sinoatrial node impulses through the severely remodeled atrial myocardium. They concluded that this pathophysiology differs from human AF, which is triggered by ectopic foci originating near the pulmonary veins. It is important to note, however, that the study by Fu et al. (2022) used a whole-heart cardiomyocyte-specific *Lkb1*-CKO allele (via crossing aMHC-Cre with *Lkb1*<sup>fl/fl</sup> mice). Thus, ventricular dysfunction, whether subclinical or overt, could have independently contributed to atrial myopathy due to volume and pressure overload.

One major limitation of the studies by Ozcan et al. (2015) and Fu et al. (2022) is that the *Lkb1*-CKO mice developed LV systolic dysfunction secondary to loss of *Lkb1* in the ventricles, which might have promoted the development of AF in these mice. To address this, Hulsurkar et al. (2021) knocked out *Lkb1* selectively in the atria (*Lkb1*-aKD) by injecting an adeno-associated virus (AAV) containing atrial natriuretic factor (ANF) promoter-driven Cre expression into 5-day-old *Lkb1*<sup>FL/FL</sup> mice (Hulsurkar et al. 2021). At six weeks, the AF incidence was 40% in *Lkb1*-aKD mice (vs 0% in controls, Fig. 2A). At 12 weeks, AF incidence was 100% in *Lkb1*-aKD mice (vs 0% in controls, Fig. 2A). AF

duration and AF burden were also statistically significantly greater in *Lkb1*-aKD mice vs controls, with the magnitude of differences increasing with age. Importantly, 12-week-old *Lkb1*-aKD mice had normal LV EF, end-diastolic diameter, and end-systolic diameter. Moreover, this study showed that the *Lkb1*-aKD mice develop AF that progresses with time, as well as atrial fibrosis, atrial dilation, and reduced atrial function, which closely mimics the human pathophysiology. Altogether, atrial-specific *Lkb1* KO mice are a robust model of spontaneous AF without a concomitant and potentially confounding ventricular phenotype.

Using the same *Lkb1*-aKD model, Moreira et al. (2020) studied the anti-fibrotic role of atrial paracrine calcitonin signaling in AF. Loss of atrial calcitonin signaling in *Lkb1*-aKD mice increased atrial fibrosis by ~ 2.5-fold compared to *Lkb1*-aKD mice with intact atrial calcitonin signaling, showing that the *Lkb1*-aKD mice could be used as a model to study the role of other genes in AF progression. Electrical remodeling also seems to play a role in spontaneous AF seen in mice with loss of *Lkb1*, as evidenced by the decreases in *Nav1.5*, *Cacna1h*, and *Kcnq1* expression observed prior to atrial structural remodeling (Kim et al. 2015).

Altogether, the loss of *Lkb1* in the heart is a robust and well-characterized model of AF recapitulating many of the essential electrocardiographic and morphologic findings seen in human AF. Evidence points toward substrate remodeling (e.g., fibrosis, delayed/impaired intra-atrial conduction) as the major AF mechanism, with unclear evidence for a role for ectopy in this model.

## CREm-Tg overexpression

cAMP-response element (CRE) and transcription factors of the CREB/CREm family function in the heart to maintain sarcoplasmic reticulum calcium ATPase 2a (SERCA2a) and beta-1 adrenergic receptors expression. While protective under physiologic conditions, CREm-mediated cAMP signaling becomes pathologic in diseases such as HF due to overstimulation and overexertion of the heart. In 2005, Muller et al. (2005) described hemizygous transgenic mice with cardiomyocyte-specific expression of a human cardiac CREm isoform (CREM-1b C-X). CREm-overexpressing transgenic (CREm-Tg) mice displayed atrial enlargement at seven weeks and AF with rapid ventricular response at eight weeks-of-age. CREm-Tg mice also demonstrated increased maximum rates of LV contraction, 32% greater beta-1 adrenergic receptor density, and 127% higher ventricular SERCA2a expression. Expression of phospholamban and RyR2 binding partners calsequestrin and junctin was unchanged. Interestingly, phospholamban phosphorylation was decreased at Ser16 and Thr17, targets of beta-1 adrenergic signaling and CaMKII, respectively. This finding was hypothesized to be a compensatory mechanism to attenuate the CREm-mediated pathologic increases in LV function.

In 2018, Stumpel et al. (2018) built upon the study by Muller et al. (2005) by designing homozygous transgenic CREm-Tg mice (Stumpel et al. 2018). This was accomplished by mating pairs of hemizygous transgenic mice and identifying homozygous transgenic mice via Southern blotting and real-time polymerase chain reaction. Transgene DNA content was roughly two-fold higher in homozygous compared to heterozygous CREm-transgenic

mice. AF incidence was 50% by 6 weeks-of-age and 100% by 10-weeks-old (Fig. 2B). At 11 weeks-of-age, treatment with the Vaughn-Williams class III anti-arrhythmic esmolol reduced overall HR and increased HR variability, reflective of the response seen in humans. However, treatment of 6–7-week-old mice with class Ic anti-arrhythmic, flecainide failed to convert the mice back into sinus rhythm, indicative of potential RyR2 and/or sodium channel dysregulation (Kryshtal et al. 2021; Yang et al. 2016). Morphologically, homozygous mice had mild ventricular hypertrophy while hemizygous mice exhibited no hypertrophy. However, no differences in ventricular collagen content were reported in either CREM-overexpressing strain compared to WT mice. While the pathophysiologic mechanism of AF in CREM-transgenic mice is not fully understood, one hypothesis is that atrial dilatation occurs secondary to systolic LV failure as LV hypertrophy was seen by 14 weeks-of-age (Stumpel et al. 2018). However, this cannot fully explain the atrial dilatation as atrial dilatation occurred prior to ventricular dysfunction, and end-diastolic aortic and LV pressures were not increased (Muller et al. 2005).

Thus, ectopy in the form of EADs and calcium mishandling likely plays a role in CREM-mediated spontaneous AF. Indeed, Kirchhof et al. (2013) demonstrated in the atria of > 15-week-old CREM-Tg heterozygous mice that interatrial conduction block with concomitant SR calcium leak is associated with decreased SR calcium load despite normal SERCA2a, phospholamban, and RyR2 protein levels. Similar findings have been demonstrated in ventricular cardiomyocytes by Schulte et al. (2016), i.e., accelerated decay of the calcium transient, mediated by increased NCX and SERCA2a transport. Increased NCX transport was associated with prolongation of the action potential and EADs. Complementing these findings, Li et al. (2014) demonstrated that enhanced atrial SR calcium leak in CREM-Tg mice was partially mediated by hyperphosphorylation of RyR2 at Serine (Ser)2814, mediated by calcium/calmodulin-dependent protein kinase II (CaMKII) and an important factor in AF initiation (Chelu et al. 2009). Importantly, Li et al. (2014) demonstrated that enhanced RyR2-mediated SR calcium leak not only provided an AF trigger but also resulted in calcium-dependent fibrotic and hypertrophic remodeling mediated via the calcineurin-nuclear factor of activated T cells (NFAT)-regulator of calcineurin (RCAN) pathway. Crossing *CREM-Tg* mice with mice carrying a mutated RyR2 phosphorylation site (Ser2814Ala) resulted in abrogation of fibrosis, indicating a fundamental role of RyR2 phosphorylation in calcium-dependent remodeling. Indeed, NFAT activity is increased in the atria of patients with chronic AF, and NFAT inhibition abrogates atrial ectopy and spontaneous AF in CREM-Tg mice, likely mediated by attenuated atrial remodeling and normalized calcium handling (Ni et al. 2021).

In addition to ectopy and calcium-dependent remodeling, inflammation has been shown to play a causal role in spontaneous CREM-mediated AF. At 7 months (~ 30 weeks) of age, double-mutant (DM) CREM-Tg/NLRP3<sup>-/-</sup> mice exhibited no spontaneous AF compared to 67% spontaneous AF incidence in CREM-Tg single mutant mice. (Yao et al. 2018) LA dilatation was concomitantly reduced in DM mice, indicating that the pro-arrhythmogenic effects of NLRP3 are, at least in part, mediated by atrial remodeling. Altogether, calcium mishandling seems central to the pathophysiology of the CREM-Tg model of spontaneous AF as dysregulated calcium provides the ectopic triggers and pathologic atrial remodeling required for AF triggering and propagation. In addition, enhanced NLRP3 inflammasome

activation appears to play a role in AF development, while the role of fibrosis is less certain in this model.

### Ace-Tg overexpression

The renin–angiotensin–aldosterone system (RAAS) is central to the pathophysiology of many cardiovascular diseases such as HF and hypertension. Increased RAAS activation is the major pathophysiologic mechanism driving systolic HF due to angiotensin II-mediated vasoconstriction and aldosterone-mediated salt and water retention. Most drugs that portend a mortality benefit in systolic HF antagonize RAAS (Stolfo and Savarese 2019). Increased RAAS is also linked with human AF, and treatment of systolic HF with RAAS blockers decreases risk of new-onset AF by 21–50% (Nair et al. 2014). To further explore the role of RAAS in AF, Xiao et al. (2004) created homozygous transgenic ACE overexpression under the  $\alpha$ MHC promoter (ACE-Tg). Consistent with unchanged renal and vascular ACE expression, ACE-Tg mice had an ~ 8.5% reduction in BP compared to WT controls (111.3 mmHg control vs 101.8 mmHg ACE-Tg). Mild atrial enlargement was observed starting at two weeks-of-age, progressing to severe atrial enlargement by three weeks-of-age. These findings occurred concomitantly with increased risk of sudden death despite a normal electrolyte profile. Ventricular size and function were also normal, suggesting that ventricular dysfunction was not the major cause of AF. Ambulatory EKGs were used to investigate the increased incidence of sudden death and revealed low QRS voltages, prolonged PR interval, and an 86% incidence (6/7) of spontaneous AF among ACE-Tg mice (Fig. 2C). By 10–16 weeks old, atrial weight was three-fold greater in ACE-Tg mice compared to controls; however, only focal atrial fibrosis was observed with no ventricular fibrosis. Altogether, the study by Xiao et al. (Xiao et al. 2004) demonstrated that spontaneous AF in mice with cardiac-specific ACE overexpression likely results, in part, from atrial myopathy with slowed conduction.

Other studies have explored AF mouse models of RAAS overactivity. While these studies have looked at inducible AF, the histologic, electrophysiologic, and molecular findings provide insight into the underlying pathophysiological role of RAAS dysregulation in the ACE-Tg mouse model of spontaneous AF. Han et al. (Han et al. 2020) demonstrated that subcutaneous angiotensin II infusion at 2,000 ng/kg/min for three weeks results in LA dilatation, fibrosis, and inflammation in 8-to-10-week-old mice. Evidence for ectopy in the pathophysiology of RAAS-mediated AF in mice comes from a study of cardiac-specific angiotensin II receptor type 1 (AT1R) overexpression (Demers et al. 2022). Six-month-old mice had a 60% reduction in inward sodium current and 50% reduction in LTCC current compared to controls. Similar to prior studies, AT1R overexpressing mice had delayed atrial conduction time with prolonged PR interval despite normal APD. Altogether, pathologic atrial structural remodeling seems to be central to the pathophysiology of the ACE-Tg mouse model of spontaneous AF, with evidence that ion channel dysregulation may provide additional ectopic electrical triggers.



## Cardiac-specific *Tnf- $\alpha$* -Tg overexpression

Saba et al. (Saba et al. 2005) created heterozygous cardiomyocyte-specific transgenic mice overexpressing *Tnf- $\alpha$*  under the  $\alpha$ MHC promoter (*Tnf- $\alpha$* -Tg). By 10 week-of-age, *Tnf- $\alpha$* -Tg mice had a 20-fold greater atrial collagen deposition by histology, concomitant with bi-atrial dilation. These changes occurred prior to ventricular enlargement, which occurred at 6–9 months-of-age. Spontaneous AF by telemetry was observed starting at 5 months-of-age, with 77% incidence in *Tnf- $\alpha$* -Tg mice (mean age  $7 \pm 4$  months old) compared to 10% in the control mice (Fig. 2D). Interestingly, mean heart rate (HR) was lower in Tg mice over 3 months old. These results were driven by Tg mice with spontaneous AF, indicating likely slowed atrial conduction perhaps mediated via decreased connexin 40 expression (Sawaya et al. 2007). Indeed, *Tnf- $\alpha$* -Tg mice had prolonged PR, QT, and QRS intervals, and optical mapping demonstrated reentrant atrial tachycardia with variable AV conduction in response to intra-atrial pacing (Saba et al. 2005). APD was similar between *Tnf- $\alpha$* -Tg mice and controls. In addition to atrial structural remodeling, slowed conduction, and reentrant-prone substrate, Saba et al. (Saba et al. 2005) demonstrated evidence for calcium mishandling in the pathogenesis of spontaneous AF in the *Tnf- $\alpha$* -Tg mice. While diastolic intracellular calcium did not differ between Tg and control mice, atrial cardiomyocytes from six-month-old *Tnf- $\alpha$* -Tg mice had reduced calcium transient amplitude and prolonged calcium transient decay, indicative of impaired SR calcium release and reuptake, respectively. Moreover, spontaneous calcium transients were three times more likely *Tnf- $\alpha$* -Tg atrial cardiomyocytes, indicating that DADs likely provide a trigger for the spontaneous AF in *Tnf- $\alpha$* -Tg mice. A role of systemic *Tnf- $\alpha$*  in AF is evidenced by the attenuation in AF after inhibition of soluble *Tnf- $\alpha$*  in a mouse model of intense exercise (Lakin et al. 2019). Altogether, the *Tnf- $\alpha$* -Tg mouse develops spontaneous AF due to ectopy in the form of DADs, which propagate to form a reentrant circuit in the setting of TNF- $\alpha$ -mediated abnormal atrial structure, conduction, and fibrotic remodeling.

## Other AF mouse models with reported spontaneous AF

*Ankyrin B KO*: Mohler et al. (Mohler et al. 2003) reported a loss-of-function mutation in the adapter protein, ankyrin B, on chromosome 4q25–27 (E1425G) found in patients presenting with long QT syndrome, sinus bradycardia, and early-onset AF. Ankyrin<sup>±</sup> mice were generated and exhibited bradycardia with high heart-rate variability and intermittent AV dissociation on ECG (Mohler et al. 2003). No structural heart defects were seen in ankyrin<sup>±</sup> mice. Cardiomyocytes isolated from ankyrin<sup>±</sup> mice had delayed calcium transient decay and increased calcium transient amplitude—likely mediated by loss of the Na<sup>+</sup>/K<sup>+</sup>-ATPase in a manner similar to the mechanism of digoxin. In response to 1  $\mu$ M isoproterenol, ankyrin<sup>±</sup> cardiomyocytes exhibited DADs and EADs, likely mediated due to RyR2 hyperactivity. Altogether this study Mohler et al. (Mohler et al. 2003) demonstrates a role of ectopy in AF in ankyrin<sup>±</sup> mice.

Cunha et al. (Cunha et al. 2011) further explored atrial function in ankyrin<sup>±</sup> mice. Telemetry revealed spontaneous AF with bradycardia in ankyrin<sup>±</sup> mice. Ankyrin<sup>±</sup> atrial cardiomyocytes had reduced APD likely mediated by reduced LTCC and NCX currents. The ventricular cardiomyocyte APD was unchanged. There were no changes in inward

sodium current or outward potassium current in ankyrin<sup>±</sup> cardiomyocytes. Studies have also implicated CaMKII-mediated RyR2 phosphorylation at Ser2814 in AF development in ankyrin<sup>±</sup> mice as inhibition of CaMKII attenuates the abnormal EP phenotype in cardiomyocytes from ankyrin<sup>±</sup> mice (DeGrande et al. 2012).

Altogether, loss-of-ankyrin increases AF susceptibility due to ectopy and a reentrant-prone substrate characterized by abbreviated APD likely predisposing to DADs.

### **Junctin overexpression**

Junctin is a transmembrane protein located on the SR that modulates RyR2 activity and regulates SR calcium release (Hong et al. 2002). To study the role of junctin in excitation–contraction coupling, Hong et al. (Hong et al. 2002) transgenically overexpressed canine cardiac junctin in mice (junctin-Tg). Six-to-eight-week-old junctin-Tg mice had biventricular and bi-atrial dilation with fibrosis and thinning of the atrial walls. Thrombi were found in both atria, indicative of stasis likely secondary to AF. Indeed, six-to-eight-week-old junctin-Tg mice had lower HR compared with WT controls, and their ECGs showed spontaneous AF. Mice died from HF between nine and 11 weeks-of-age. These findings, together with prolonged APD and increased LTCC current in junctin-Tg atrial cardiomyocytes, suggest that junctin overexpression causes AF due to atrial structural and electrical remodeling, creating a reentrant-prone substrate. The role of ectopy (i.e., DADs and EADs) is less clear in this model. While the AF incidence was not reported in the study, the relative early-onset and severity of the electrophysiological findings indicate that this model induces a severe AF phenotype.

### **Sarcoplipin (SLN) KO**

SLN, like phospholamban, is a negative regulator of SERCA2a activity expressed in cardiac and skeletal muscle (Bhupathy et al. 2007), present more in the atria than in ventricular myocardium. SLN is phosphorylated via CaMKII and inhibits SERCA2a-mediated SR calcium uptake. Right atrial SLN levels have been shown to be decreased in chronic AF, with the degree of decrease proportional to right atrial pressure (Uemura et al. 2004). To this end, Xie et al. (Xie et al. 2012) created homozygous cardiac *Slnc*<sup>-/-</sup> mice. Atrial cardiomyocytes from *Slnc*<sup>-/-</sup> mice had increased SR calcium content and calcium transients. APD was prolonged, mediated by a greater forward mode NCX current. DADs were observed along with increasing LTCC current. Phosphorylation of RyR2 at Ser2808 and Ser2814 were both increased in the atria of *Slnc*<sup>-/-</sup> mice. No atrial hypertrophic effect was observed, but trichrome staining revealed atrial fibrosis. ECGs were normal at five months-of-age, and spontaneous AF was seen starting at 12 months-of-age with 66.7% incidence (Xie et al. 2012). Altogether, calcium mishandling plays a key role in AF in *Slnc*<sup>-/-</sup> mice, with fibrosis and prolonged APD creating a reentry-prone substrate.

### **RhoGTPase inhibition and Muscle-restricted putative coiled-coil protein (MURC) overexpression**

The Rho GTPase family, which includes RhoA, Rac1, and Cdc42, mediates pressure and volume overload cardiac hypertrophy (Clerk and Sugden 2000). Overexpression of RhoA in the heart results in HF with concomitant SA and AV nodal dysfunction (Sah et al. 1999). On

the other hand, inhibition of cardiac-specific RhoA signaling is also pathologic, resulting in atrial and ventricular enlargement by 4 months-of-age in mice. Surface ECGs showed 100% incidence of first-degree AV block by 4 weeks old with normal QRS and QTc intervals. At 7 months old, atrial arrhythmias were seen in mice with deficient cardiac RhoA signaling—transient sinus arrest (60%), sinus bradycardia (60%), atrial tachycardia (60%), and AF (20%). RhoA-deficient mice also had prolonged atrial ERP, indicative of impaired atrial recovery from electrical triggers. While the association between deficient cardiac RhoA signaling and AF is rather weak (20% incidence), atrial arrhythmias like resulted from an AF-prone substrate characterized by hypertrophy and slowed conduction.

Building upon these findings, Ogata et al. (Ogata et al. 2008) studied MURC, a molecule localized to the Z-line of sarcomeres that functions as a positive modulator of Rho GTPase signaling in the heart via augmentation of atrial natriuretic peptide (ANP) expression (Ogata et al. 2008). MURC was overexpressed in the murine heart by cloning mouse MURC into the 5' untranslated region of the  $\alpha$ MHC promoter (Ogata et al. 2008). At 13 weeks-of-age, MURC-overexpressing mice had atrial enlargement with intra-atrial thrombi and ventricular wall thinning. Fibrosis was seen histologically in the atria and ventricles bilaterally. Echocardiography revealed LV systolic dysfunction, likely mediated by ventricular fibrosis. At nine weeks-of-age, surface ECGs revealed that 16.7% of the MURC-overexpressing mice had AF, 8.3% had 3<sup>rd</sup> degree AV block, and 75% had prolonged PR intervals. At 20 weeks-of-age, 42% of MURC-overexpressing mice had either AF or AV block. Altogether, the RhoA GTPase pathway predisposes to primarily via structural remodeling, as evidenced by fibrosis, atrial dilatation, and concomitant LV systolic dysfunction. A role for calcium mishandling and other ectopic triggers remains elusive in this model.

### Phosphoinositide 3-kinase (PI3K) KO

PI3K is a key regulator of physiologic cardiac hypertrophic growth (McMullen et al. 2003) that has been shown to inhibit pathologic G-protein coupled receptor signaling cascades (McMullen et al. 2007) and exhibits crosstalk with the AMPK metabolic stress pathway (Walkowski et al. 2022; Harada et al. 2012). Inhibition of PI3K exacerbates systolic dysfunction in a mouse model of dilated cardiomyopathy (DCM) (McMullen et al. 2007). Based on several studies demonstrating clinical and biochemical evidence of a link between PI3K and AF (van der Hooft et al. 2004; Kampinga et al. 2007), Pretorius et al. (Pretorius et al. 2009) crossed a transgenic mouse model of DCM (Yamamoto et al. 2003) with cardiac-specific transgenic mice expressing a dominant negative mutant of PI3K (Pretorius et al. 2009). Expression of the mutant PI3K transgene in the DCM Tg mice increased the PR interval and resulted in spontaneous AF occurred in 40% of 4.5-month-old mice. Atrial fibrosis was 5.5-fold higher by histology in the double transgenic mice compared to WT controls. Atrial expression of potassium channels *Kcnv2*, *Kcnd3*, and *Kcnj2* were also decreased in the DCM/PI3K-Tg mice compared to DCM-Tg and WT control mice. Human atrial samples showed lower PI3K in patients who developed paroxysmal AF following cardiothoracic surgery—indicating that lower PI3K levels, whether due to aging or other pathology, may predispose patients to post-operative AF. It is important to note with this model of spontaneous AF, however, that HF exacerbation could have caused and/or contributed to spontaneous AF. Nonetheless, aged (15-month-old) DCM single Tg mice did

not exhibit AF nor the decrease in *Kcnj2* and *Kcnv2* seen in the DCM/PI3K-Tg mice. Thus, PI3K seems to play an independent role in AF generation apart from the HF also seen with loss of PI3K. Altogether, Pretorius et al. (Pretorius et al. 2009) provides evidence that the increase in AF susceptibility may be mediated through changes in substrate characterized by structural, electrical, and fibrotic remodeling.

### Rac1 overexpression

AF is associated with oxidative stress, in which nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity in the left atrium plays an important role (Dudley et al. 2005). Rac1 is a Rho GTPase regulator of NADPH oxidase activity that has been shown to mediate oxidative stress and hypertrophic responses in the heart (Custodis et al. 2006). To this end, Adam et al. (Adam et al. 2007) created homozygous mice with overexpression of active Rac1 under control of the alpha-MHC promoter (Adam et al. 2007). Because concentric ventricular hypertrophy was seen as early as three weeks old, transverse aortic constriction (TAC) was performed to differentiate the atrial effects of Rac1 activation secondary to ventricular hypertrophy from the atrial effects of Rac1 overexpression itself. Homozygous mice overexpressing Rac1 developed spontaneous AF, with incidences of 44% at 10 months and 75% at 16 months. Atrial fibrosis was increased in Rac1-Tg mice compared to WT mice who underwent TAC; atrial fibrosis was more pronounced than ventricular fibrosis. AF patient samples similarly showed greater Rac1 protein expression. Altogether, spontaneous AF in Rac1 overexpressing mice was mediated by substrate remodeling, as evidenced by atrial fibrosis, with potential contribution from concomitant ventricular dysfunction.

### Nucleoporin (NUP) deletion

Mutations in chromosome 5p13, which encodes the *NUP155* gene, have been linked to autosomal-recessive AF (Oberti et al. 2004). NUP155 encodes for nucleoporin, a component of the nuclear pore complex. One mutation in the 5p13 region, R391H, co-segregates with AF, and has been shown to decrease nuclear envelope permeabilization (Zhang et al. 2008). To further explore this genetic evidence, Zhang et al. (Zhang et al. 2008) made heterozygous NUP155 deletions in mice. Loss of NUP155 resulted in spontaneous AF with shortened APD. AF pathophysiology in this model was hypothesized to be due to attenuation of mRNA nuclear export of key atrial genes such as calcium handling proteins and ion channels, as evidenced by the shortened APD seen in NUP155<sup>±</sup> atrial cardiomyocytes.

### Micro-RNAs (miR)

The role of micro-RNAs as therapeutic and diagnostic targets is an emerging field of research in cardiovascular disease (Romaine et al. 2015). miR-382, which targets the alpha 1c and beta-1 subunits of the cardiomyocyte LTCC, is upregulated in AF patients with rheumatic heart disease (Lu et al. 2010). To explore the implications of this finding, Lu et al. (Lu et al. 2010) transgenically overexpressed miR-328 in the hearts of mice under the  $\alpha$ MHC promoter. Spontaneous AF was observed in 100% of Tg mice starting at 28 days-of-age. Consistent with *Cacna1c* and *Cacnb1* being targets of miR-328, the LTCC current was decreased in miR-328 Tg mice, resulting in shortened atrial APD. On the other hand, potassium currents were unaltered. Altogether, miR-328 induces spontaneous AF due

to atrial electrical remodeling, characterized by shortened APD secondary to decreased LTCC current. Evidence for the role of fibrotic remodeling comes from a study of miRs collected from exosomes of pericardial fluid that linked miR-328 with cardiac fibrosis (Liu et al. 2020).

miR-208a is encoded by an intron of the alpha-myosin heavy chain (*Myh6*) and has been implicated in stress-related cardiac growth and remodeling (van Rooij et al. 2007). Callis et al. (Callis et al. 2009) demonstrated that miR-208a overexpression causes ventricular hypertrophy and dilatation with decreased EF by three months of age. miR-208a overexpression also resulted in AV block in four-month-old mice. In contrast to overexpression, loss of miR-208a, which also prolonged the PR interval, resulted in spontaneous AF in 80% of mice with concomitant decreased connexin 40 expression. These seemingly contradictory results of miR-208a overexpression and knockout could perhaps be explained by the fact that miR-208a directly targets key cardiac transcription factor GATA4, and therefore both overexpression and loss-of-function results in pathologic GATA4 dysregulation. Altogether, the spontaneous AF seen in miR-208a<sup>-/-</sup> mice results from structural remodeling and aberrant conduction.

**Na<sub>v</sub>1.5 overexpression**—Based on the observation that gain-of-function variants in *SCNA5* are associated with increased AF incidence, a prior study generated Tg mice overexpressing human Na<sub>v</sub>1.5-F1759A, which causes a persistent sodium current (Wan et al. 2016). Na<sub>v</sub>1.5-F1759A Tg mice exhibited biatrial enlargement by 1 month old, with histologic evidence of fibrosis as well as ventricular dilation and systolic HF by 3–4 months of age (Wan et al. 2016). Na<sub>v</sub>1.5-F1759A mice had prolonged QT intervals, indicative of persistent inward sodium current causing APD prolongation. Spontaneous AF was observed in 25% of mice by 5–6 weeks of age. By 10 weeks of age, spontaneous AF incidence was reported in 80% of mice (Wan et al. 2016). A more recent study of the same model reported that oxidative stress due to mitochondrial reactive oxygen species could a mechanistic driver of AF in the Na<sub>v</sub>1.5-F1759A mouse model (Avula et al. 2021). Phosphorylation of RyR2 by protein kinase A and CaMKII at Ser2808 and Ser2814, respectively, as well as calcium spark frequency were greater in Na<sub>v</sub>1.5-F1759A mice compared to controls (Avula et al. 2021). These changes were abrogated after catalase overexpression, providing evidence of causality for mitochondrial reactive oxygen species in the Na<sub>v</sub>1.5-F1759A mouse model (Avula et al. 2021).

## Conclusion

While there are many mouse models of AF, only a handful of these models exhibit spontaneous AF as the majority of mouse models require supraphysiological stimuli to uncover (inducible) AF. Among the spontaneous mouse models of AF, atrial dilatation and fibrosis seem to be common to most. Indeed, atrial dilatation and fibrosis are two of the most well-known risk factors for AF in humans and mice (Li et al. 2021; Schotten et al. 2003). In line with known AF pathophysiology (Heijman et al. 2014), calcium mishandling is also prevalent among spontaneous AF mouse models, particularly the CREM-Tg model. The most robust and well-studied models of spontaneous AF, *Lkb1*-CKO and CREM-Tg, exhibit characteristics of fibrosis and calcium dysregulation, with the *Lkb1*-KO model appearing

to be driven more by fibrosis and CREM-Tg driven more by calcium mishandling. Taken together, spontaneous mouse models of AF are useful to study the effects of molecular, biochemical, and genetic manipulation on electrophysiologic phenotype and for gene therapy in targeting specific arrhythmia-promoting pathways (Dobrev and Wehrens 2018). Results must be interpreted in context, however, as human AF differs from mouse AF. For instance, human AF arises from ectopic foci near the pulmonary veins (Khan 2004), but this finding is not typically seen in mouse AF, which could be due to phenotyping limitations (Schuttler et al. 2020). Thus, understanding the underlying pathophysiology of spontaneous mouse models of AF is key to enable clinical translation.

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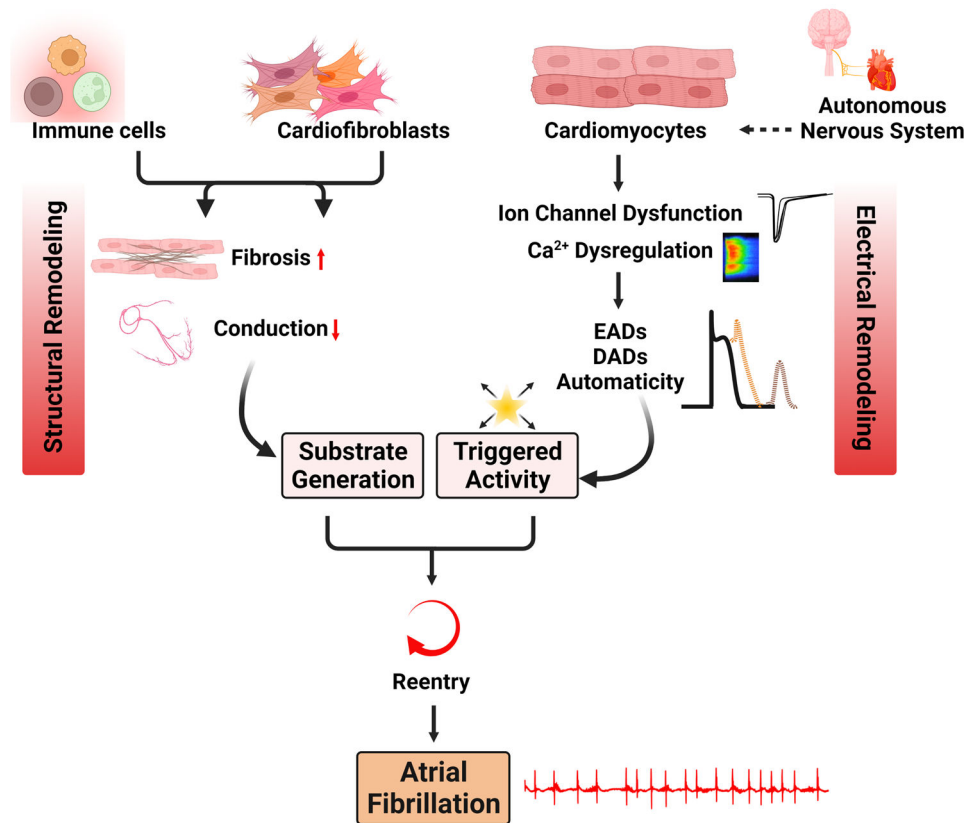


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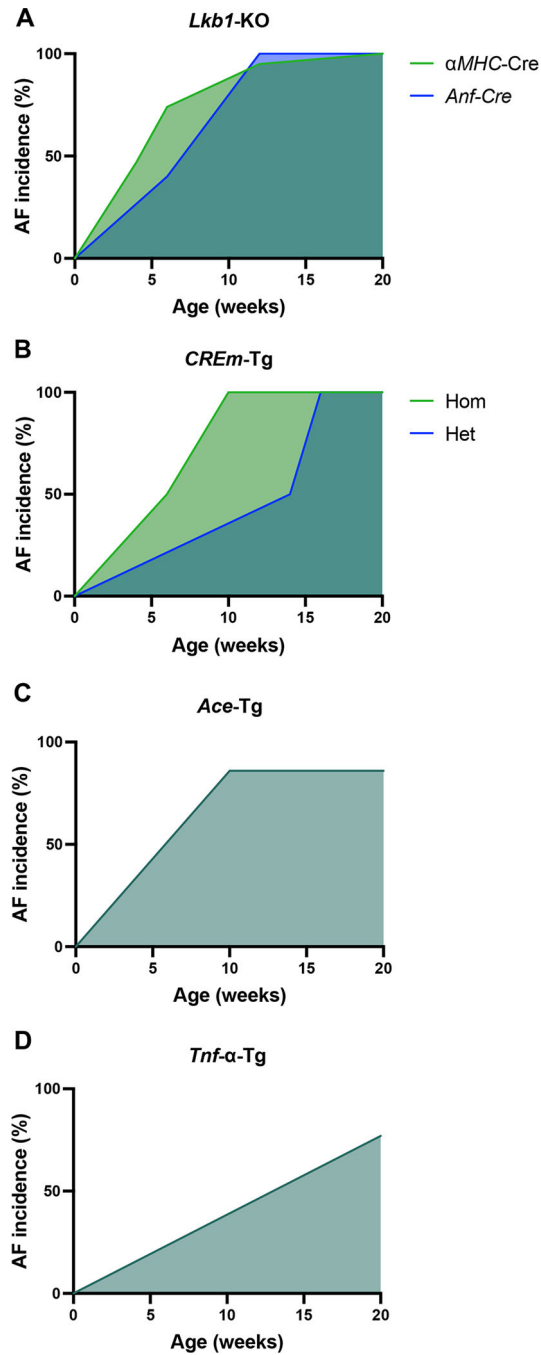
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**Fig. 1.** Major AF mechanisms present in mouse models of spontaneous AF. Altered substrate and ectopic electrical activity are the two major mechanisms. Altered substrate is characterized by fibrosis and slowed conduction. Ectopy is characterized by EADs, DADs, and automaticity, which arise due to ion channel dysfunction and calcium dysregulation. *AF* atrial fibrillation, *Ca<sup>2+</sup>* calcium, *DADs* delayed afterdepolarizations, *EADs* early afterdepolarizations



**Fig. 2.** AF incidence by age in the *Lkb1*-CKO, *CREm*-Tg, *Ace*-Tg, and *Tnf-α*-Tg mouse models. Incidence of spontaneous AF is plotted against age for *Lkb1*-KO (Ozcan et al. 2015; Hulsurkar et al. 2021) (A), *CREm*-Tg (Muller et al. 2005; Stumpel et al. 2018) (B), *ACE*-Tg (Xiao et al. 2004) (C), and *Tnf-α*-Tg (Saba et al. 2005) (D). Age was plotted through 20 weeks for all models. *Ace* angiotensin-converting enzyme; *Anf* atrial natriuretic factor; *CREm* cyclic AMP-responsive element modulator; Hom homozygous; Het heterozygous;

*Lkb1* liver kinase B1;  $\alpha$ -MHC myosin heavy chain; KO knockout; Tg transgenic; *Tnf- $\alpha$*  tumor necrosis factor alpha

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Table 1

Mechanisms contributing to AF development in mouse models of spontaneous AF

Model	Altered substrate			Ectopy			References
	Atrial fibrosis/ dilatation	ERP changes	Slowed conduction	DADs	EADs	Calcium mishandling	
<i>Lkb1</i> -KO	+++	-	+	Not reported	Not reported	Not reported	(Ikeda et al. 2009; Ozcan et al. 2015; Hulsurkar et al. 2021)
<i>CREM</i> -Tg	++	+	+	+	+	+++	(Muller et al. 2005; Stumpel et al. 2018; Kirchhof et al. 2013; Schulte et al. 2016; Li et al. 2014)
<i>Acc</i> -Tg	++	+	+	Not reported	Not reported	+	(Xiao et al. 2004; Demers et al. 2022)
<i>Tnf-<math>\alpha</math></i> -Tg	+++	+	+	+	Not reported	+	(Saba et al. 2005)
Ankyrin B KO	-	+	+	++	++	++	(Mohler et al. 2003; Cunha et al. 2011)
Junctin-Tg	++	+	Not reported	Not reported	Not reported	+	(Hong et al. 2002)
Sarcoplipin KO	+	+	Not reported	+	Not reported	++	(Xie et al. 2012)
MURC-Tg	++	+	+	Not reported	Not reported	Not reported	(Sah et al. 1999) (Ogata et al. 2008)
PI3K KO	++	+	Not reported	Not reported	Not reported	Not reported	(Pretorius et al. 2009)
<i>Rac1</i> -Tg	+++	Not reported	Not reported	Not reported	Not reported	Not reported	(Adam et al. 2007)
Nucleoporin KO	Not reported	+	Not reported	Not reported	Not reported	+	(Zhang et al. 2008)
miR-328-Tg	+	++	Not reported	Not reported	Not reported	++	(Lu et al. 2010; Liu et al. 2020)
miR-208a KO	+	Not reported	+	Not reported	Not reported	Not reported	(Callis et al. 2009)

*ACE* angiotensin-converting enzyme, *CREM* cyclic AMP-responsive element modulator, *DADs* delayed afterdepolarizations, *EADs* early after-depolarizations, *ERP* effective refractory period, *Lkb1* liver kinase B1, *KO* knockout, *miR* microRNA, *MURC* Muscle-restricted putative coiled-coil protein, *PI3K* Phosphoinositide 3-kinase, *Tg* transgenic, *TNF- $\alpha$*  tumor necrosis factor alpha