Electrophysiological and molecular mechanisms of paroxysmal atrial fibrillation

Stanley Nattel[1–3]* *and Dobromir Dobrev*[1,3]*

Abstract | Atrial fibrillation (AF) is an extremely prevalent arrhythmia that presents a wide range of therapeutic challenges. AF usually begins in a self-terminating paroxysmal form (pAF). With time, the AF pattern often evolves to become persistent (nonterminating within 7 days). Important differences exist between pAF and persistent AF in terms of clinical features, in particular the responsiveness to antiarrhythmic drugs and ablation therapy. AF mechanisms have been extensively reviewed, but few or no Reviews focus specifically on the pathophysiology of pAF. Accordingly, in this Review, we examine the available data on the electrophysiological basis for pAF occurrence and maintenance, as well as the molecular mechanisms forming the underlying substrate. We first consider the mechanistic insights that have been obtained from clinical studies in the electrophysiology laboratory, noninvasive observations, and genetic studies. We then discuss the information about underlying molecular mechanisms that has been obtained from experimental studies on animal models and patient samples. Finally, we discuss the data available from animal models with spontaneous AF presentation, their relationship to clinical findings, and their relevance to understanding the mechanisms underlying pAF. Our analysis then turns to potential factors governing cases of progression from pAF to persistent AF and the clinical implications of the basic mechanisms we review. We conclude by identifying and discussing questions that we consider particularly important to address through future research in this area.

[1]*Department of Medicine and Research Center, Montreal Heart Institute and Université de Montréal, 5000 Belanger Street East, Montreal, Quebec H1T 1C8, Canada.*
[2]*Department of Pharmacology and Therapeutics, McGill University, 3655 Promenade Sir William Osler, Montreal, Quebec H3G 1Y6, Canada.*
[3]*Institute of Pharmacology, West German Heart and Vascular Center, University Duisburg-Essen, Hufeland Strasse 55, 45122 Essen, Germany.*

Correspondence to S.N.
stanley.nattel@icm-mhi.org

**These authors contributed equally to this work.*

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Atrial fibrillation (AF) is the most common arrhythmia and is associated with increased cardiovascular morbidity and mortality, which are mediated predominantly by stroke, myocardial infarction, and heart failure[1,2]. Present AF therapy with drugs has moderate efficacy and many limitations, particularly proarrhythmia and bleeding complications[3]. Ablation procedures have emerged as efficient therapeutic options, but they cannot be applied to the majority of this very large patient population[4]. Although maintenance of sinus rhythm (rhythm control) seems desirable, clinical studies have not demonstrated superiority of rhythm over rate control, possibly because of the low efficacy of currently used rhythm-control drugs[3]. Therefore, a clear unmet need exists for a better mechanistic understanding of the molecular mechanisms of AF, in order to foster the development of novel mechanism-based treatment approaches.

The molecular mechanisms underlying AF are poorly understood. Independent of the underlying cause, which can be clinically very diverse, abnormal impulse formation (focal ectopic activity) and re-entry are thought to be the two major determinants of AF initiation and maintenance[5,6] (FIG. 1), although the precise clinical correlates of these fundamental mechanisms are not uniformly validated and are somewhat controversial[7]. Re-entry requires an arrhythmogenic substrate and an initiating trigger. The likelihood of re-entry formation is determined by the tissue properties of conduction and refractoriness, with abnormal conduction (slowing and/or local block) and short refractoriness increasing the likelihood of re-entry. Ectopic activity results primarily from Ca^{2+}-handling abnormalities that cause early and delayed afterdepolarizations (EADs and DADs, respectively)[5,6]. Changes in atrial structure and function that result from genetic and epigenetic factors, cardiac and noncardiac diseases, ageing, and AF itself, constitute atrial remodelling that can increase the likelihood of both re-entry and ectopic activity[8].

AF often presents first in a paroxysmal form (pAF; defined by episodes that last <7 days and terminate spontaneously), then in persistent (duration of episodes >7 days), and finally long-standing persistent

Key points

- Paroxysmal atrial fibrillation (pAF) has discrete clinical features from persistent forms, including greater relative pathophysiological importance of the pulmonary vein sleeves and greater susceptibility to both medical and ablation therapy
- Evidence indicates that both focal ectopic activity and re-entry can have a role in pAF, and that the pulmonary veins have structural and electrophysiological features that favour both mechanisms
- The mechanisms underlying pAF are likely to vary between patients, depending on factors such as genetic background, cardiovascular risk factors, and concomitant heart disease
- Ca^{2+}-dependent triggered activity seems to underlie atrial ectopy in pAF, and has complex underlying molecular mechanisms that increase both cellular Ca^{2+} load and the leakiness of the sarcoplasmic reticulum Ca^{2+}-release channel (ryanodine receptor)
- Molecular mechanisms promoting re-entrant activity in patients with pAF include ionic properties (such as larger left atrial inward-rectifier background current) and structural properties (such as atrial fibrosis)
- Fairly little attention has been paid in the literature to the specific mechanistic basis of pAF; more work is needed to provide insights with translational potential

AF forms[6,9]. Clinically, some patients with pAF progress to persistent/permanent AF, whereas others never develop persistent forms. In addition, some patients present initially with persistent AF[10]. The reason for these differences is not clear. Although there are limitations to the standard clinical classification as pAF, persistent AF, and long-standing persistent AF, pAF does have distinct clinical properties and behaviours that justify its distinction from the other forms[4]. For example, pAF is much more susceptible to control by ablation procedures than persistent AF[11], and the longer the AF episodes last, the more resistant they become to therapy[12,13]. Research over the past decades has expanded our understanding of the processes involved in the initiation, maintenance, and progression of AF; however, many important issues related to the spontaneous initiation and termination of AF that lie at the core of pAF pathophysiology remain poorly understood. On searching the literature, we were unable to identify a single Review article that focused specifically on the mechanisms underlying pAF. Accordingly, in this Review, we summarize the available information on the electrophysiological and molecular factors governing pAF. We consider data obtained in clinical studies as well as results obtained in human atrial tissue samples and clinically relevant animal models, deal with the mechanisms of progression of pAF to persistent AF forms, discuss the potential clinical implications, and suggest future research directions in this underexplored area.

Insights into pAF from clinical studies

Clinical electrophysiology

Investigators in the Haïssaguerre Laboratory first noted the contribution of the pulmonary vein (PV) cardiomyocyte sleeves in pAF[14]. They initially described an important role for focal ectopic activity in the PVs of nine patients with drug-resistant pAF associated with frequent atrial premature complexes and runs of atrial tachycardia[14]. Seven of 13 ectopic sources in these patients were located in the PVs. Subsequently, in a seminal paper, they noted that 94% of focal sources in 45 patients were located in the PVs[15]. These patients had frequent episodes of AF lasting for minutes or hours; AF paroxysms occurred unpredictably during electrophysiological study (EPS), and were difficult to initiate with programmed stimulation or drug provocation. The properties of these arrhythmias were most consistent with focal ectopic activity, and ablation at the site of arrhythmia origin suppressed AF[15]. Follow-up observations showed that AF tended to recur because of emergence of new PV sources, leading to a variety of ablation procedures seeking to isolate all PVs to prevent recurrence[16]. In addition, sources in thoracic veins other than the PVs (such as venae cavae, coronary sinus, and ligament of Marshall) can also contribute to the initiation of AF[16]. Numerous subsequent studies indicated the critical importance of PV isolation in preventing pAF recurrence, with approaches aiming to ensure enduring PV isolation greatly increasing the long-term success of ablation therapy[17].

Whereas early studies emphasized the importance of PVs as a source of focal ectopic activity, subsequent work pointed to the PVs as a privileged zone for re-entry. A case report described features typical of re-entry (entrainment, overdrive termination, induction by EPS) in an isolated PV of a patient with pAF[18]. Atienza *et al.* studied the effects of adenosine in a series of patients with pAF, noting that adenosine accelerated dominant frequencies during AF, particularly of the fastest zones at the junction of the PV and left atrium[19]. Given that adenosine accelerates rotor activity by increasing inward-rectifier K^+-current but slows automatic tachycardias[20], these results point to PV re-entry sustaining AF in these patients. Electroanatomical abnormalities are prominent in PVs of patients with pAF, with lower electrogram voltages, slowed conduction, and shorter effective refractory periods (ERPs), indicating a re-entry-prone substrate; AF induction during PV–ERP testing occurred in patients with AF, but not controls[21]. Qualitatively similar remodelling has also been observed in the left atrium of patients with AF[22]. Electroanatomical disturbances were significant in both groups, but tended to be greater in persistent AF than pAF. Another study performed in patients with 'lone' pAF (no cardiovascular diseases, pulmonary disease, hyperthyroidism, diabetes mellitus, or hypertension) demonstrated that patients with pAF have larger atrial volumes, longer ERP, longer bi-atrial conduction time, slower conduction velocity, a greater proportion of fractionated electrograms, longer corrected sinus node recovery time, and lower voltage, pointing to electroanatomical abnormalities in non-PV regions of these patients[23]. In addition, impulse-propagation disturbances including decremental conduction are common in patients with drug-resistant pAF, with decremental conduction observed in 76%, versus ectopic activity during EPS in only 26%[24]. In contrast to pAF, which is often terminated by PV isolation alone, additional ablation lesions are often required to terminate persistent AF, pointing to the emergence of non-PV sources of arrhythmogenesis[25].

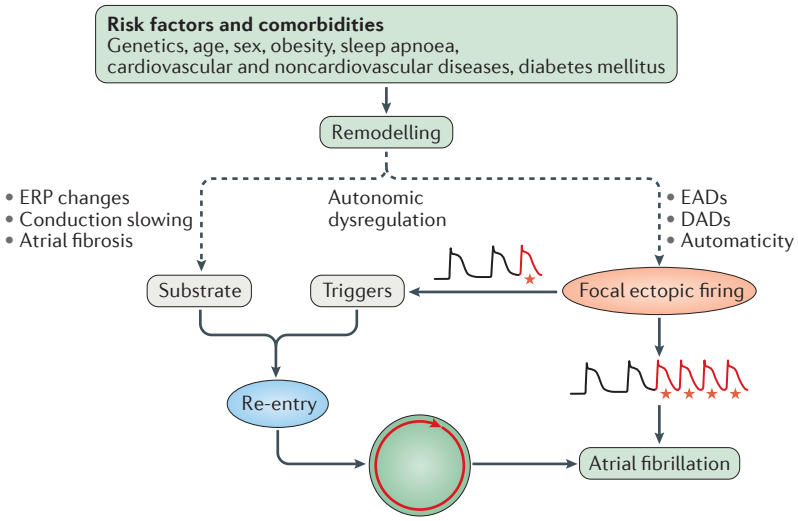

Figure 1 | **General mechanistic concepts of atrial fibrillation.** Atrial fibrillation is initiated and maintained by focal ectopic firing and re-entry. Re-entry requires a substrate and a trigger to initiate it. The trigger must initiate unidirectional block, and generally involves premature or rapid ectopic activity. Numerous risk factors and cardiovascular and noncardiovascular diseases alter atrial function and structure (remodelling), increasing the likelihood of ectopic firing and re-entry. The remodelled substrate includes effective refractory period (ERP) changes, conduction slowing, and atrial fibrosis. Focal ectopic firing is believed to result from abnormal automaticity, and early and delayed afterdepolarizations (EADs and DADs).

Noninvasive observations

Ambulatory monitoring has revealed apparent triggering atrial premature complexes at the onset of >95% of self-terminating pAF episodes in 90 patients[26]. Heart-rate variability analysis suggested increased vagal tone in the 5 min preceding AF onset[26]. Another study similarly reported evidence for increased vagal tone immediately preceding pAF onset in 77 patients[27]. 'Vagal' AF — based on onset during typical vagal triggers (sleep in >96%, postprandial in >96%) — is present in about 27% of patients with pAF, whereas 'adrenergic' AF — based on onset during contexts like emotion and exercise — is present in only 7% of patients with pAF[28]. Atrial premature complexes increase in frequency and prematurity immediately before pAF, defined by the researchers as "recurrent attacks of generally self-terminating AF" (REF. 29).

Genetic analysis

Analysis of gene variants associated with pAF can provide additional insights into underlying mechanisms. Gain-of-function K^+-channel subunit mutations, usually accompanied by clear evidence of markedly accelerated repolarization in terms of short QT syndrome, have been described as the probable cause of pAF in at least four publications[30–33]. The involved genes and related K^+-currents are *KCNJ2* (I_{K1})[30,32], *KCNE1* (I_{Ks})[31], and *KCNH2* (I_{Kr})[33]. Given that increased K^+ current abbreviates refractoriness and promotes re-entry, while tending to reduce automaticity, these cases of pAF are likely to be caused by re-entry. This notion is supported by the clinical data available, with (in the two reports with sufficient information to judge) AF requiring cardioversion for termination and, when terminated, not re-emerging for prolonged periods (several months)[30,33]. Zhang *et al.* reported a family with a mutation in nuclear pore complex protein (nucleoporin) Nup155, which presented with AF and sudden death in early childhood[34]. Ablation of *Nup155* in mice resulted in abbreviated ERP and action potential duration (APD), and spontaneous development of AF probably mediated by re-entry[34]. By contrast, several other reports suggest that abnormal automaticity can be central to the pathophysiology of pAF. Kazemian *et al.* described a patient with catecholaminergic polymorphic ventricular tachycardia owing to a ryanodine receptor (RyR2) mutation, who presented with self-terminating AF paroxysms with exercise that were subsequently controlled by β-adrenergic blockade[35]. A loss-of-function junctophilin mutation was identified in a patient with juvenile-onset pAF, and found to enhance RyR2 Ca^{2+} leak, DADs, and AF in a mouse model[36]. Investigators in another study noted downregulation of the RyR2-targeting microRNA (miR)-106b-25 cluster in pAF[37]. Ablation of miR-106b-25 in mice increased RyR2 protein expression and Ca^{2+} leak, and caused atrial premature complexes and AF inducibility *in vivo* that were suppressed by an RyR2-stabilizing drug (K201, also known as JTV519)[37]. Therefore, ion-channel mutations give valuable insights into the mechanisms underlying pAF; however, they are a rare cause of pAF in the population[38].

Translational considerations

The electrophysiological mechanisms underlying pAF are complex, but consideration of clinical presentations allows the identification of different forms with distinct mechanisms[39]. Some patients present with discrete sustained episodes that self-terminate in about 70% of cases if AF onset was recent (≤48 h)[40] and, when terminated, the pAF will not recur for weeks or months[39]. These patients are likely to have a re-entrant mechanism maintaining AF. Other individuals present with many AF episodes, all self-terminating and of fairly short duration, sometimes with periods of rapid irregular atrial tachycardias, as in the patients initially described by Haïssaguerre and colleagues[14,15]. These patients are likely to have focal sources involving ectopic automatic mechanisms. Many patients with pAF probably have a combination of automatic and re-entrant mechanisms. The vast majority of patients with pAF respond well to PV isolation, suggesting a central participation of the PVs in both automatic and re-entrant mechanisms.

FIGURE 2 shows schematically the features that make the PVs vulnerable sites for both automatic and re-entrant activity. The anatomical properties of PVs involve strands of cardiomyocytes projecting over the veins[41]. These strands are poorly coupled to atrial tissue, an arrangement that reduces the current-sink from quiescent atrial tissue and promotes automatic activity, as occurs in the sinus node[42]. An additional feature that contributes to PV automaticity is the ion-current make-up of PV cardiomyocytes, with smaller inward-rectifier K^+-current (I_{K1}) and shorter APD than in left atrial cells[39,43]. The structural properties of PV cardiomyocyte sleeves also favour

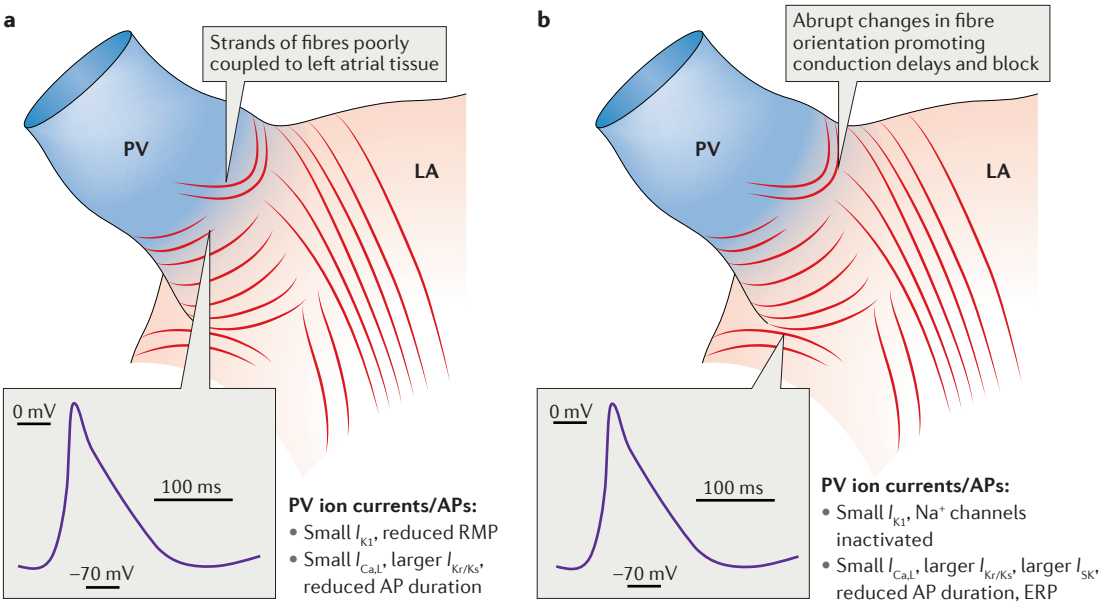

Figure 2 | **Mechanisms of atrial fibrillation initiation at the pulmonary veins.** The pulmonary veins (PVs) are vulnerable sites for both **a** | ectopic and **b** | re-entrant activity. AP, action potential; ERP, effective refractory period; $I_{Ca,L}$, L-type Ca^{2+}-current; I_{K1}, inward-rectifier K^+-current; I_{Kr}, rapid delayed-rectifier K^+-current; I_{Ks}, slow delayed-rectifier K^+-current; I_{SK}, small-conductance Ca^{2+}-dependent K^+-current; LA, left atrium; RMP, resting membrane potential.

re-entry, with abrupt changes in fibre orientation that lead to localized conduction slowing that can become quite prominent with rapid rates or premature activation[44]. The ion-current properties of PVs make re-entry more likely by abbreviating ERP (APD shortening owing to increased rapid [I_{Kr}] and slow [I_{Ks}] delayed-rectifier K^+-currents and small-conductance Ca^{2+}-dependent K^+-current [I_{SK}] and reduced L-type Ca^{2+}-current [$I_{Ca,L}$]) and slowing conduction (via Na^+-current inactivation owing to membrane depolarization from reduced I_{K1})[39,43,45,46]. The role of the small PV I_{K1} in re-entry is complex — it might favour re-entry by promoting conduction abnormalities through I_{Na} inactivation, but can also be antiarrhythmic via reduced excitability[47].

Molecular mechanisms of pAF

Tissue samples from patients with pAF

Molecular basis of ectopic activity. Focal ectopic activity can result from enhanced or abnormal atrial automaticity and EADs or DADs (FIG. 3). The mechanisms have been reviewed in detail previously[5,6,8]. DADs and associated triggered activity are currently believed to be the principal basis of ectopic atrial firing, and the central event causing DADs is abnormal spontaneous release of Ca^{2+} from the sarcoplasmic reticulum (SR; the main subcellular storage organelle) during diastole. The released Ca^{2+} is exchanged for extracellular Na^+ in a 1:3 ratio by the Na^+–Ca^{2+} exchanger, causing a net inward flow of positive ions that depolarizes the cell[5,6,8]. When large enough, the resulting DAD can reach threshold and cause spontaneous firing.

Studies in atrial tissue, primarily obtained from the right atrial appendage, suggest that focal ectopic activity and re-entry can both contribute to the initiation and maintenance of repetitive AF episodes in patients with pAF. FIGURE 3 and FIGURE 4 summarize our current understanding of the molecular mechanisms in pAF. FIGURE 3 summarizes the available data on the molecular mechanisms that make patients with pAF more susceptible to spontaneous ectopic activity than control individuals. Right atrial cardiomyocytes from patients with pAF have an increased incidence of spontaneous SR Ca^{2+}-release events, depolarizing transient-inward currents (I_{ti}), and corresponding DADs and triggered activity compared with patients in sinus rhythm[36,48]. The underlying molecular substrate involves increased SR Ca^{2+} load and RyR2 dysregulation leading to enhanced diastolic SR Ca^{2+} leak. The increased SR Ca^{2+} load is likely to result from protein kinase A-dependent hyperphosphorylation of the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) 2a inhibitor phospholamban, relieving phospholamban inhibition of SERCA2a, and leading to an enhancement of SR Ca^{2+} uptake[48] (FIG. 3). Increased protein levels of calreticulin, a SR-located calsequestrin-like Ca^{2+}-binding protein, might also contribute to the increased SR Ca^{2+} load in pAF[49].

In contrast to patients with long-standing (>6 month duration) persistent AF[50], RyR2 dysregulation in patients with pAF does not result from RyR2 hyperphosphorylation by increased calcium/calmodulin kinase II (CaMKII) activity[48]. Protein kinase A phosphorylation of RyR2 is reduced in patients with pAF, without a corresponding change in the expression of protein kinase A subunits[48]; alterations in the interactome of protein phosphatase type 1 is likely to contribute to reduced Ser2808 phosphorylation in pAF[51]. These findings point to distinct molecular mechanisms of potentially proarrhythmic RyR2 dysregulation in patients with pAF compared with those with long-standing persistent AF. Accordingly, RyR2 dysregulation in patients with

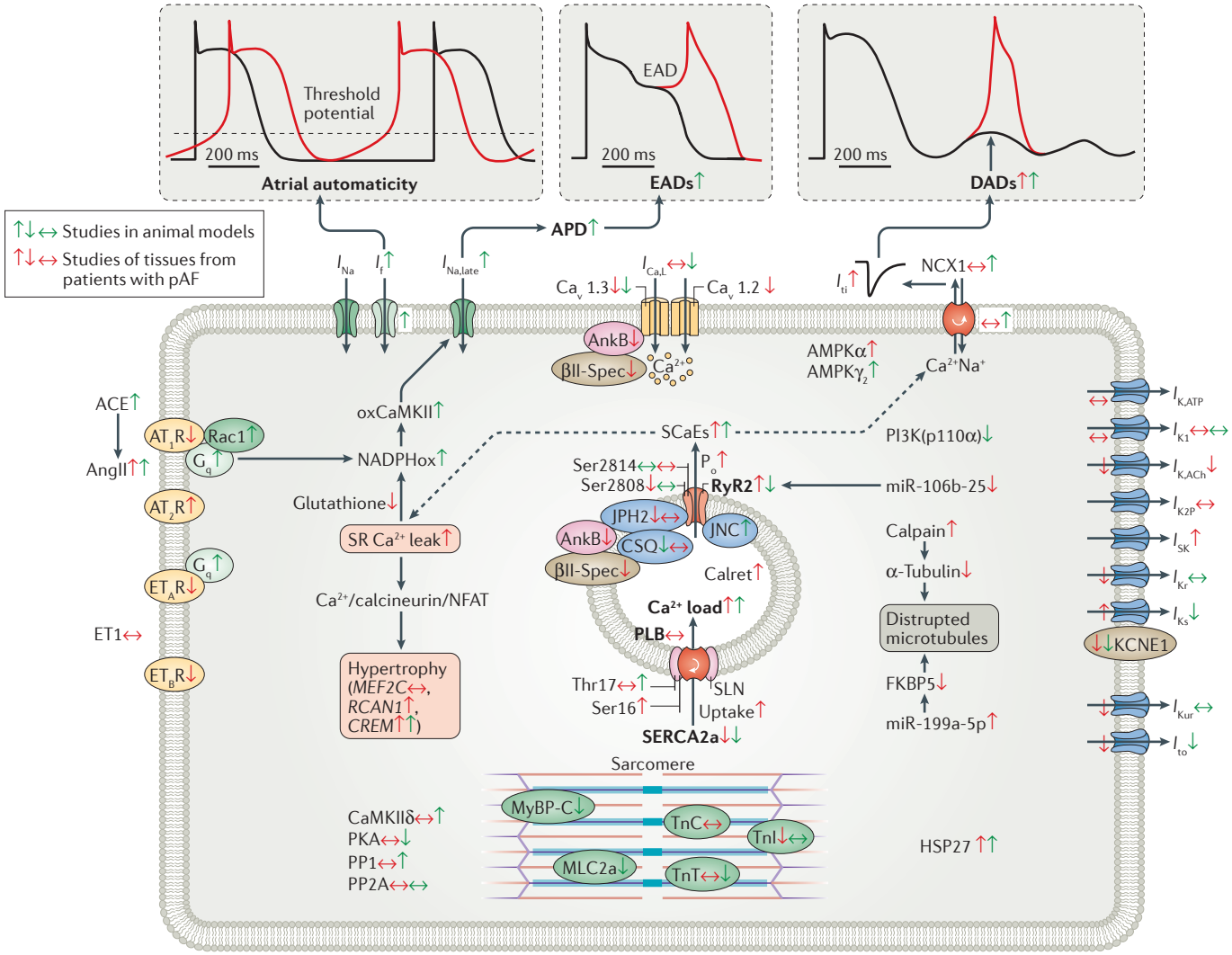

Figure 3 | **Molecular mechanisms of focal ectopic firing in paroxysmal atrial fibrillation (pAF).** Abnormal atrial automaticity, early and delayed afterdepolarizations (EADs and DADs) are the major determinants of focal ectopic firing. Abnormal automaticity might result from an upregulation of hyperpolarization-activated cyclic nucleotide-gated (HCN)-mediated current (I_f). EADs generally occur in the setting of prolonged action potential duration (APD), for example, with loss of repolarizing K^+-currents, or an excessive 'late' component of non-inactivating Na^+-current ($I_{Na,late}$). Angiotensin II (AngII)-mediated increase in NADPH oxidase (NADPHox) with subsequent oxidation (activation) of calcium/calmodulin dependent protein kinase II (oxCaMKII) contributes to increased $I_{Na,late}$. DADs arise from abnormal sarcoplasmic reticulum (SR) Ca^{2+} leak and diastolic SR Ca^{2+}-release events (SCaEs) through ryanodine receptor type 2 channels (RyR2) and are promoted by increased SR Ca^{2+} load and RyR2 dysfunction. SCaEs activate sodium–calcium exchanger type 1 (NCX1), producing a transient-inward current (I_{ti}) that causes membrane depolarization. If the DAD reaches threshold, it causes a triggered ectopic action potential. Enhanced SR Ca^{2+} load results from increased uptake by sarco/endoplasmic reticulum Ca^{2+}-ATPase type 2a (SERCA2a) owing to increased levels of the Ca^{2+}-binding protein calreticulin (Calret) and hyperphosphorylation (dissociation) of inhibitory phospholamban (PLB) from SERCA2a. Decreased miR-106b-25-mediated suppression of RyR2 protein expression along with altered composition of RyR2 partners within the macromolecular RyR2 complex increase channel open probability (P_o) resulting in increased diastolic Ca^{2+} leak through RyR2. SR Ca^{2+} leak also activates the Ca^{2+}-dependent phosphatase calcineurin, which dephosphorylates (activates) the nuclear factor of activated T-cells (NFAT) to increase transcription of the prohypertrophic genes (*CREM*, *MEF2C*, and *RCAN1*). Calpain-mediated degradation of myofilament proteins and microtubules causes cardiomyocyte contractile dysfunction. Focal ectopic firing can also arise from micro re-entrant circuits that, at the macroscopic level, cannot be distinguished from EAD/DAD-mediated triggered activity. Red (human) and green (nonhuman animal) arrows indicate increase, decrease, or no change in either mRNA/protein levels or protein function. βII-Spec, βII-Spectrin; ACE, angiotensin-converting enzyme; AMPKα, AMP-dependent protein kinase catalytic subunit α; $AMPK\gamma_2$; AMP-dependent protein kinase regulatory subunit γ_2; AnkB, ankyrin B; AT_1R, type 1 angiotensin II receptor; AT_2R, type 2 angiotensin II receptor; $Ca_v1.2$ and $Ca_v1.3$, pore-forming α_{1C} and α_{1D} $I_{Ca,L}$-channel subunits; CSQ, calsequestrin; ET1, endothelin 1; ET_AR, endothelin 1A receptor; ET_BR, endothelin 1B receptor; FKBP5, FK506-binding protein 5; G_q, G_q protein; HSP27, heat-shock protein 27; $I_{Ca,L}$, L-type Ca^{2+}-current; I_{K1}, inward-rectifier K^+-current; I_{K2P}, two-pore channel domain K^+-current; $I_{K,ACh}$, ACh-activated inward-rectifier K^+-current; $I_{K,ATP}$, ATP-dependent K^+-current; I_{Kr}, rapid component of delayed-rectifier K^+-current; I_{Ks}, slow component of delayed-rectifier K^+-current; I_{Kur}, ultra-rapid delayed-rectifier K^+-current; I_{Na}, Na^+-current; I_{SK}, small conductance Ca^{2+}-dependent K^+-current; I_{to}, transient-outward K^+-current; JNC, junctin; JPH2, junctophilin 2; KCNE1, potassium voltage-gated channel subfamily E member 1; MLC2a, myosin light chain 2a; MyBP-C, myosin-binding protein C; PI3K(p110α), phosphoinositide 3-kinase, catalytic subunit p110α; PKA, protein kinase A; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; Rac1, Rho small GTP-binding protein; Ser16, PKA phosphorylation site of PLB; Ser2808, PKA phosphorylation site of RyR2; Ser2814, CaMKII phosphorylation site of RyR2; SLN, sarcolipin; Thr17, CaMKII phosphorylation site of PLB; TnC, cardiac troponin C; TnI, cardiac troponin I; TnT, cardiac troponin T.

pAF involves increased RyR2 channel subunit expression and greater single-channel open probability, which would increase the likelihood and size of spontaneous SR Ca^{2+}-release events[48]. Loss of the miR-106b-25 cluster increases the protein levels of RyR2 without changing corresponding mRNA, and miR-106b-25 knock-out mice have increased atrial ectopy and susceptibility to pacing-induced AF[37,52]. Given that levels of miR-106b-25 are reduced by ~50% in right atrial tissue of patients with pAF[37], reduced microRNA-106b-25 is likely to be a contributor to the atrial RyR2 protein upregulation noted in patients with pAF[37,48]. A relative deficiency of the RyR2-stabilizing protein junctophilin 2, resulting from increased RyR2 but unaltered junctophilin 2 expression, might explain the greater open probability of single RyR2 channels in patients with pAF compared with individuals

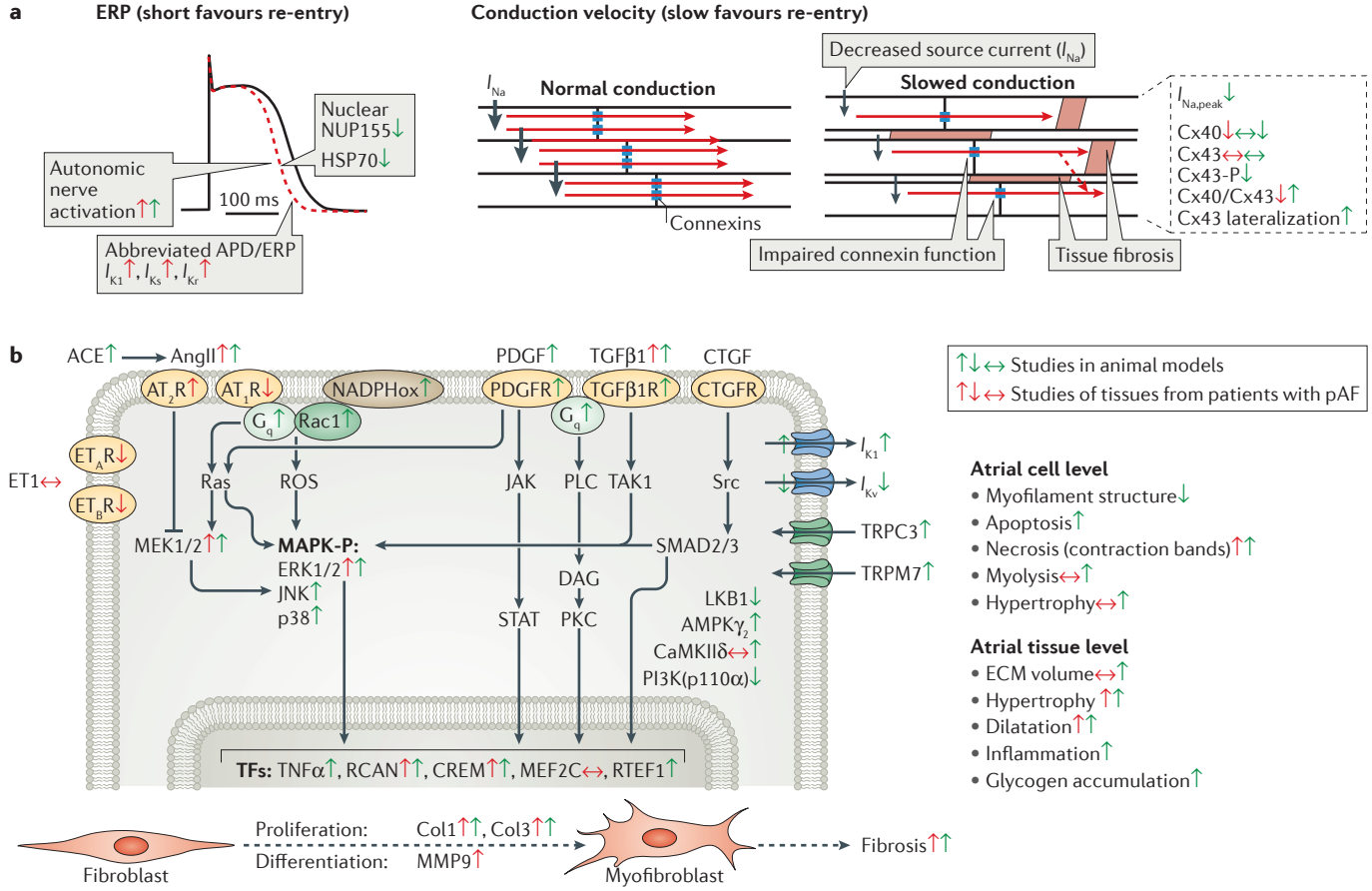

Figure 4 | **Molecular mechanisms governing re-entry in paroxysmal atrial fibrillation (pAF). a** | Functional features promoting re-entry include abbreviation of action potential duration (APD)/effective refractory period (ERP) owing to an increase in repolarizing K^+ currents, and impaired conduction owing to reduction in source Na^+ current (I_{Na}) and dephosphorylation/reduced expression of connexins (Cxs) resulting in disrupted cell–cell contact. **b** | Structural abnormalities at the atrial cell level (myolysis, apoptosis, necrosis) and the tissue level (dilatation, hypertrophy, inflammation, and fibrosis) also promote re-entry by producing heterogeneous conduction and obstacles to conduction. Atrial fibrosis results from increased proliferation and differentiation of fibroblasts into collagen-secreting myofibroblasts, a process that can result from activation of angiotensin II (AngII), transforming growth factor-β1 receptor (TGFβ1R), platelet-derived growth factor receptor (PDGFR), and connective tissue growth factor receptor (CTGFR), and downstream signalling via mitogen-activated ERK kinase 1/2 (MEK1/2), mitogen-activated protein kinase (MAPK) (extracellular signal-regulated MAPK [ERK], c-Jun N-terminal kinase [JNK], p38 MAPK [p38]), signal transducers and activators of transcription (STAT), protein kinase C (PKC), and SMA-related and MAD-related proteins (SMAD2/3) kinase pathways leading to enhanced gene transcription of proliferation-inducing and differentiation-promoting signals. Red (human) and green (nonhuman animal) arrows indicate increase, decrease, or no change in either mRNA/protein levels or protein function. ACE, angiotensin-converting enzyme; AT_1R, type 1 angiotensin II receptor; AT_2R, type 2 angiotensin II receptor; AMPKγ_2; AMP-dependent protein kinase regulatory subunit γ_2; CaMKIIδ, calcium/calmodulin-dependent protein kinase IIδ; Col1, collagen 1; Col3, collagen 3, CREM, cAMP-response element modulator; CTGF, connective tissue growth factor; DAG, diacylglycerol; ECM, extracellular matrix; ET1, endothelin 1; ET_AR, endothelin 1A receptor; ET_BR, endothelin 1B receptor; HSP70, heat-shock protein 70; G_q, G_q protein; I_{K1}, inward-rectifier K^+-current; I_{Kr}, rapid component of delayed-rectifier K^+-current; I_{Ks}, slow component of delayed-rectifier K^+-current; I_{Kv}, voltage-dependent K^+-current; JAK, Janus kinase; LKB1, liver kinase B1 (also known as serine/threonine-protein kinase STK11); MEF2C, myocyte-specific enhancer 2c; MMP9, metalloproteinase 9; NADPHox, NADPH oxidase; NUP155, nuclear pore complex protein Nup155; PLC, phospholipase C; PDGF, platelet-derived growth factor; PI3K(p110α), phosphoinositide 3-kinase catalytic subunit p110α; Rac1, Rho small GTP-binding protein; Ras, small GTPase protein; RCAN, regulator of calcineurin (also known as calcipressin 1); ROS, reactive oxygen species; RTEF1, α_1-adrenergic signalling mediating transcription enhancer factor 1-related protein (also known as transcriptional enhancer factor TEF 3); Src, proto-oncogene tyrosine-protein kinase; TAK1, TGFβ-activated kinase 1; TF, transcription factor; TGFβ1, transforming growth factor-β1; TNFα, tumour necrosis factor α; TRPC3, transient receptor potential cation channel subfamily C member 3; TRPM7, transient receptor potential cation channel subfamily M member 7.

in sinus rhythm[36]. βII-Spectrin regulates the localization of cytoskeletal and plasma membrane/SR protein complexes, including the Na^+–Ca^{2+} exchanger, RyR2, and ankyrin B[53], and its protein levels are ~50% lower in patients with pAF, who also have a ~60% decrease in ankyrin B protein expression[54,55]. Both of these changes are likely to contribute to pAF-related RyR2 dysregulation, although the detailed mechanisms of SR Ca^{2+} leak in pAF are still incompletely understood.

Molecular basis of re-entry. The main functional determinants of AF are refractory period (primarily driven by APD), excitability, and conduction properties including conduction heterogeneity and block[5,6]. Decreases in refractoriness, conduction abnormalities, and local conduction blocks that stabilize re-entry circuits favour re-entry and are all implicated in AF (FIG. 4). The current knowledge of the underlying molecular basis of these changes in pAF is summarized in FIGURE 4.

The molecular determinants of re-entry susceptibility in patients with pAF are less well studied than those of spontaneous ectopy. Indices of classical electrical remodelling, such as shortening of APD, are absent in right atrial tissue of patients with pAF[48,56,57]. In multicellular preparations of right atrial appendages, upstroke velocity, amplitude, duration at 20%, 50%, and 90% of repolarization of action potential, and resting membrane potential were similar in sinus rhythm and patients with pAF[57], and were similarly unchanged in right atrial cardiomyocytes from patients with pAF[48,56]. Consistent with these findings, $I_{Ca,L}$ (as indicated by both current amplitude and expression of its pore-forming α_{1C} subunit)[48,52], I_{K1} (REF. 58), two-pore domain K^+-current I_{K2P} (TASK-1)[56], and voltage-gated K^+-channels $K_v1.1$ (REF. 59) were not altered in right atrial cardiomyocytes of patients with pAF (FIG. 3). Preliminary data point to increased small-conductance Ca^{2+}-dependent K^+-current in patients with pAF[60]. Molecular studies showed that the expression of $K_v4.3$ (pore-forming subunit of the transient-outward K^+-current I_{to}), $K_v1.5$ (pore-forming subunit of the ultra-rapid delayed-rectifier K^+-current I_{Kur}), KCNH2 (also known as HERG; the pore-forming subunit of I_{Kr}), and KCNE1 (also known as minK; the regulatory subunit of I_{Ks}) were reduced; $K_{ir}6.2$ (pore-forming subunit of ATP-sensitive K^+-channels) was unchanged; and K_vLQT1 (pore-forming subunit of I_{Ks}) was increased in right atrial tissue of patients with pAF compared with sinus rhythm[61,62]. Although corresponding ion-current recordings are not available, the changes in ion currents might offset each other, explaining the similar action potential shape in patients with pAF and individuals in sinus rhythm. In individuals with short QT syndrome, however, mutation-caused increases in either I_{Kr} or I_{Ks} currents lead to pAF suggesting that an increase in these currents is sufficient to produce pAF in humans[31,33].

Inward-rectifier K^+-currents are important determinants of functional re-entry and proarrhythmic high-frequency rotors[19]. I_{K1} is increased in the left atrium, but not the right atrium, of patients with pAF, without concomitant increases in channel-subunit expression[58,61,62]. This finding corresponds to the clinical observation that the left atrium seems to be more central to sustaining AF than the right atrium. Agonist-activated inward-rectifier K^+-current $I_{K,ACh}$ is larger in the right atrium than the left atrium from individuals in sinus rhythm, but is decreased in the right atrium of patients with pAF owing to a reduction in underlying $K_{ir}3.1$ and $K_{ir}3.4$ subunits[58,61,62]. Given that $I_{K,ACh}$ is the major effector of vagal nerve stimulation, the capacity of parasympathetic activation to abbreviate the atrial action potential might be limited in patients with pAF. Furthermore, $K_{ir}3.4$, but not $K_{ir}3.1$, is regulated by intracellular Na^+ concentration, resulting in a Na^+-dependent increase in agonist-activated $I_{K,ACh}$, and this Na^+-dependent regulation might be lost in pAF owing to the large reduction in the Na^+-sensitive subunit $K_{ir}3.4$, which should further reduce $I_{K,ACh}$ at fast rates with increased intracellular Na^+ concentration[63]. However, increased total inward-rectifier K^+-current (I_{K1} plus $I_{K,ACh}$) in the left atrium of patients with pAF[58] might stabilize re-entrant rotors by shortening APD and hyperpolarizing the resting membrane potential, which is consistent with the capacity of genetically enhanced I_{K1} to cause pAF in individuals with short QT syndrome[30,32].

In addition to leading to DADs and triggered activity, SR Ca^{2+} leak in pAF might stimulate Ca^{2+}-dependent pathways that alter gene transcription, predisposing to pAF-promoting atrial hypertrophy, atrial dilatation, and atrial fibrosis. Indeed, a combined transcriptomic analysis and miR microarray study in right atrial samples from patients with pAF identified 113 genes and 49 miRs that were differentially expressed in patients with pAF compared with individuals in sinus rhythm[64]. Numerous novel pathways and miR–mRNA regulations were also uncovered, pointing to multiple changes in gene transcription and complex atrial remodelling in pAF[64]. This study also showed that expression of many metabolism-related genes is altered[64]. Relative Thr172 phosphorylation (activation) of AMP-dependent protein kinase (AMPK) is increased in right atrial tissue from patients with pAF, and AMPK phosphorylation regulates the atrial response to metabolic stress[65]. Therefore, abnormal atrial metabolism might be an important contributor to the occurrence of AF episodes in patients with pAF.

Increased expression of regulator of calcineurin (RCAN, also known as calcipressin 1), a marker of calcineurin–NFAT signalling and hypertrophy, is noted in patients with pAF[66], suggesting activation of the calcineurin–NFAT signalling pathway. In mice with cardiac-restricted overexpression of a repressor form of the cAMP-response element modulator (CREM), chronic activation of the calcineurin–NFAT pathway is associated with reduced conduction velocity and decreased connexin 40 (also known as gap junction-α5 protein) expression as well as progressive spontaneous AF[66]. The slowing in atrial conduction and the reduction in protein expression of connexin 40 (but not connexin 43, also known as gap junction-α1 protein) in patients with pAF are consistent with this notion[23,67] (FIG. 4). The reduction in connexin 40 and the related disruption in cell–cell contacts is a potential determinant of re-entry-promoting atrial conduction abnormalities.

Table 1 | **Animal models with spontaneous development of atrial ectopy, AT, or AF**

Model	Description	Phenotype	Study
Mouse			
ACE 8/8 TG	Transgenic homozygous mutation with cardiac-restricted ACE overexpression	• Spontaneous AF • Bi-atrial hypertrophy • Atrial selective fibrosis • Atrioventricular block • Sudden death • Normal left ventricular function	Xiao *et al.* (2004)[98]
$G\alpha_q$-TG	Transgenic model with cardiac overexpression of activated $G\alpha_q$ protein	• Spontaneous AF • ↑Atrial APD • Atrial fibrosis • Left atrial dilatation • Left atrial thrombus formation • ↓Conduction velocity	Hirose *et al.* (2009)[93]
AMPK TG^{N488I}	Transgenic missense mutation model overexpressing *PRKAG2* (γ_2 subunit of AMPK)	• Spontaneous AT/AF • Cardiac hypertrophy • Cardiomyocyte hypertrophy • ↑Cardiac glycogen accumulation • Sinus bradycardia • ↓RR interval • Cardiomyopathy	Arad *et al.* (2003)[99]
LKB1 knock-out	Transgenic knock-out of cardiac-specific AMPK-activating LKB1	• Spontaneous AF • Atrial fibrosis • Bi-atrial hypertrophy • Left atrial dilatation • ↓Connexin 40 and connexin 43 • ↓AMPK activation • ↑Atrial inflammation • ↑Oxidative stress • Disrupted cardiomyocyte ultrastructure • Heart failure	Ozcan *et al.* (2015)[97]
F1759A-$Na_v1.5$-dTG	Transgenic model with conditional (doxycycline-inducible) cardiac-specific expression of mutated human sodium channel $Na_v1.5$	• Spontaneous AF • ↑Persistent Na^+ current • Normal peak Na^+ current • Atrial hypertrophy • ↑Glycogen deposition • Atrial fibrosis • Mitochondrial injury • Myofibrillar disarray • ↓Ejection fraction • ↑Bi-atrial APD_{90} • ↑RR interval • ↑Heterogeneity of APD • Normal conduction • Bi-atrial rotors • Polymorphic ventricular tachycardia	Wan *et al.* (2016)[90]
D1275N-$Na_v1.5$	Transgenic model with a missense mutation in human sodium channel $Na_v1.5$	• Spontaneous AF • ↓Peak Na^+ current • ↑Persistent Na^+ current • ↑Ventricular APD • ↑RR interval • Sinus node dysfunction • Atrioventricular block and ventricular tachycardia • Dilated cardiomyopathy • ↓Conduction velocity	Watanabe *et al.* (2011)[100]
dnPI3K-DCM	Transgenic model with cardiac-specific expression of a dominant negative mutation in phosphoinositide 3 kinase catalytic subunit p110α)	• Spontaneous AF • Atrial fibrosis • Atrial hypertrophy • Chronic left atrial thrombi • ↓HSP70 • Altered expression of multiple K^+-channels and metabolism-related genes • Dilated cardiomyopathy	Pretorius *et al.* (2009)[101]
Kcne1$^{-/-}$	Transgenic model with deletion of K^+-channel KCNQ1 protein partner KCNE1	• Spontaneous AF • ↑I_{Ks} • ↓Atrial APD • Normal atrial size and structure	Temple *et al.* (2005)[91]

Table 1 (cont.) | **Animal models with spontaneous development of atrial ectopy, AT, or AF**

Model	Description	Phenotype	Study
Mouse (cont.)			
LTCC ($\alpha1D^{-/-}$)	Transgenic model lacking the α1D subunit of L-type Ca^{2+} channel	• Spontaneous AF • ↓$I_{Ca,L}$ • ↓SR Ca^{2+} content • ↓Ca^{2+} transient amplitude	Mancarella *et al.* (2008)[146]
$K_{ir}2.1$ TG	Transgenic model with cardiac-specific overexpression of the pore-forming $K_{ir}2.1$ I_{K1} channel subunit	• Spontaneous AT/AF • Atrial hypertrophy • ↓Atrial APD/ERP • ↓QT interval • ↑$I_{Ca,L}$	Li *et al.* (2004)[92]
RTEF1 TG	Transgenic model with cardiac-specific overexpression of α_1-adrenergic signalling mediating RTEF1	• Spontaneous AT/AF • Atrial dilatation • Atrial hypertrophy • ↓Conduction velocity • ↑PP1β phosphatase • ↓Phosphorylation of connexin 40 and connexin 43	Chen *et al.* (2004)[102]
Nup155$^{+/-}$	Transgenic model with a heterozygous mutation (R391H) in nucleoporin NUP155 which reduces nuclear envelope permeability	• Spontaneous AF • Normal atrial size and structure • ↓Atrial APD • ↓HSP70 localization in nucleus • Normal HSP27	Zhang *et al.* (2008)[34]
CREM-IbΔC-X	Transgenic line with heart-directed overexpression of CREM-IbΔC-X	• Spontaneous atrial ectopy, AT, and AF • Atrial dilatation • Atrial fibrosis • Left atrial thrombi • Atrial hypertrophy • Cardiomyocyte hypertrophy • Interatrial conduction block • ↑Atrial conduction heterogeneity • ↑Atrial APD and ERP • ↓Connexin 40 • ↑SR Ca^{2+} leak • ↑CaMKII activity • ↑Calcipressin 1	• Müller *et al.* (2005)[86] • Kirchhof *et al.* (2013)[87] • Li *et al.* (2014)[66]
TG junctate 1	Transgenic line with overexpression of the SR-located Ca^{2+}-binding protein junctate 1	• Spontaneous AF • Bi-atrial hypertrophy • Intra-atrial thrombi • Atrial fibrosis • Biventricular hypertrophy • Bradycardia • ↑Atrial APD • ↑$I_{Ca,L}$ • ↓Ca^{2+} transient amplitude • ↓SR Ca^{2+} content • ↓RyR2 channel • ↓Calreticulin • ↓Phospholamban phosphorylation • ↓Troponin I phosphorylation	Hong *et al.* (2008)[94]
TG junctin	Transgenic line with cardiac-restricted overexpression of RyR2-binding partner protein junctin	• Spontaneous AF • Bradycardia • Atrial dilatation • Atrial hypertrophy • Atrial wall thinning • Atrial fibrosis • ↑Atrial APD • ↓Diastolic Ca^{2+} level • ↓Ca^{2+} transient amplitude • ↑$I_{Ca,L}$ • ↓RyR2 channel	Hong *et al.* (2002)[95]
MHCsTNF TG	Transgenic model with cardiac-specific tumour necrosis factor overexpression	• Spontaneous AF • ↓Connexin 40 • Lateralized connexin 43 distribution • Atrial and ventricular conduction disturbances • ↓PR interval • Wide QRS secondary to junctional rhythm	Sawaya *et al.* (2007)[103]

Table 1 (cont.) | **Animal models with spontaneous development of atrial ectopy, AT, or AF**

Model	Description	Phenotype	Study
Mouse (cont.)			
RacET	Transgenic model with overexpression of constitutively active Rac1 Rho GTPase	• Spontaneous AF • Atrial hypertrophy • Atrial fibrosis • ↑NADPH oxidase activity • ↓Ejection fraction	Adam *et al.* (2007)[104]
MHC-TGFcys^{33}ser	Transgenic model with cardiac-restricted overexpression of a constitutively active form of TGFβ1	• Burst pacing plus ACh followed by spontaneous recurrence of re-entrant AF • Atrial selective fibrosis • ↓Atrial APD • ↓Conduction velocity • ↑Atrial Ca^{2+} transient	Choi *et al.* (2012)[96]
Casq2$^{-/-}$	Transgenic line with homozygous knock out of (cardiac) calsequestrin 2	• Spontaneous atrial ectopy with isoprenaline plus ACh • Sinus node dysfunction • Bi-atrial hypertrophy • ↓Conduction velocity • Mild cardiomyopathy	Glukhov *et al.* (2015)[88]
Dct$^{-/-}$	Transgenic line with homozygous knock out of melanin synthesis enzyme dopachrome tautomerase	• Spontaneous AT • Normal atrial size and structure • ↑Reactive oxygen species generation • ↑Atrial ERP • Cellular early afterdepolarizations / triggered activity	Levin *et al.* (2009)[89]
Rat			
Spontaneously hypertensive rat	Long-standing arterial hypertension in spontaneously hypertensive rats	• Spontaneous AT/AF • Cardiac hypertrophy • ↓Pituitary homeobox 2 (PITX2) • ↑β-Myosin heavy chain 7 • ↓Connexin 43 • Autonomic imbalance with relative vagal hyperactivity	• Scridon *et al.* (2012)[105] • Scridon *et al.* (2015)[106]
Dog			
Chronic atrial ischaemia	Right atrial infarction owing to chronic coronary artery occlusion	• Spontaneous atrial ectopy and AT • Normal atrial APD/ERP • Atrial fibrosis • ↑Conduction heterogeneity • ↑Cellular DADs/triggered activity • ↓Diastolic Ca^{2+} level • ↓Ca^{2+} transient amplitude • ↓SR Ca^{2+} content • ↑NCX current	Nishida *et al.* (2011)[107]
VTP-induced HF	Heart failure owing to 2 weeks of VTP at 240 bpm	• Spontaneous atrial ectopy and AT • Atrial fibrosis • ↑Atrial APD/ERP • ↑Conduction heterogeneity • Cardiomyocyte hypertrophy • DADs/triggered activity • ↑Ca^{2+} transient amplitude • ↑SR Ca^{2+} content • ↑NCX current	• Yeh *et al.* (2008)[108] • Burstein *et al.* (2009)[109] • Wakili *et al.* (2010)[110]
Dogs with autonomic remodelling and sympathovagal activation	Dogs with intermittent high-rate (640 bpm) pacing for 6 days with simultaneous extrinsic and intrinsic cardiac nerve activity recording during pacing-free 24 h monitoring periods	• Spontaneous AT/AF preceded by sympathovagal activation paralleled by complex fractionated atrial electrograms • Nerve sprouting • Sympathetic hyperinnervation • ↑Cardiac norepinephrine content	• Tan *et al.* (2008)[111] • Choi *et al.* (2010)[112]
Dogs with pharmacological sympathovagal activation	Dogs with adrenergic (epinephrine) and cholinergic (ACh) stimulation	• Spontaneous AF initiation with simultaneous adrenaline and ACh perfusion	Sharifov *et al.* (2004)[113]
Pig			
Pigs with pharmacological sympathovagal activation	Pigs with adrenergic (epinephrine) and cholinergic (ACh) stimulation	• Spontaneous AF initiation with administration of ACh followed by adrenaline	Carneiro *et al.* (2015)[114]

ACE, angiotensin-converting enzyme; ACh, acetylcholine; AF, atrial fibrillation; AMPK, AMP-activated protein kinase; APD, action potential duration; AT, atrial tachycardia; CaMKII, calcium/calmodulin kinase II; CREM, cAMP-response element modulator; DAD, delayed afterdepolarization; ERP, effective refractory period; HSP, heat-shock protein; $I_{Ca,L}$, L-type Ca^{2+}-current; I_{K1}, inward-rectifier K^+-current; I_{Ks}, slow component of delayed-rectifier K^+-current; KCNE1, potassium voltage-gated channel subfamily E member 1; KCNQ1, potassium voltage-gated channel subfamily KQT member 1; LKB1, liver kinase B1 (also known as serine/threonine-protein kinase STK11); NCX, sodium–calcium exchanger; NUP155, nuclear pore complex protein Nup155; PP1β, protein phosphatase 1β; RTEF1, transcription enhancer factor 1-related protein; RyR2, ryanodine receptor 2; SR, sarcoplasmic reticulum; TG, transgenic; TGFβ1, transforming growth factor β1; VTP, ventricular tachypacing.

Activity of the Ca^{2+}-dependent protease calpain is increased in the right atrium of patients with pAF and mediates degradation of cardiac troponin I (but not cardiac troponin C or troponin T) and perhaps other proteins, contributing to disruption of cardiomyocyte structure and contractility[68,69]. Activated calpain also disturbs the microtubular structure of cardiomyocytes through derailment of α-tubulin proteostasis[70], and reduced levels of FK506-binding protein 5 (FKBP5) might also lead to microtubule destabilization[64] (FIG. 3). Oxidative stress might also contribute, because tissue levels of the major antioxidant and redox buffer glutathione are ~50% lower in left atrial tissue from patients with pAF[71]. Signs of necrosis, including contraction bands and pyknotic nuclei of cardiomyocytes, are increased in patients with pAF versus individuals in sinus rhythm, suggesting increased cardiomyocyte necrosis in pAF atria[68]. Hibernation (reduction in the number of sarcomeres owing to myolysis) was not observed[68], perhaps because expression of heat-shock protein 27 is increased in patients with pAF[72]. Overall, the observed molecular changes in cardiomyocyte structure are likely to contribute to atrial arrhythmogenesis in pAF.

Atrial fibrosis and dilatation promote re-entry and are central features of atrial structural remodelling in patients with AF[6] (FIG. 4). Angiotensin II and transforming growth factor-β1 (TGFβ1) are major profibrotic signalling molecules, with additional roles for platelet-derived and connective-tissue growth factors[6,8]. Cardiac fibrosis is usually preceded by increased tissue angiotensin II levels and activation of mitogen-activated protein kinases such as c-Jun N-terminal kinase and extracellular signal-related kinase (ERKs). In right atrial tissue of patients with pAF, TGFβ1 and angiotensin II levels are increased, although the protein levels of type 1 angiotensin II receptor are reduced by ~50%, whereas those of type 2 angiotensin II receptor are increased by ~500%[49,73,74]. Levels of endothelin 1, a prohypertrophic and profibrotic peptide, are unchanged, whereas protein expression of its endothelin A and endothelin B receptors is reduced in right atrial tissue of patients with pAF[75]. In addition, protein expression of total and phosphorylated (activated) ERK1/2 and MEK1/2 are strongly increased in patients with pAF compared with individuals in sinus rhythm[76]. These changes provide a potential molecular basis for increased proliferation of fibroblasts and their differentiation into collagen-secreting myofibroblasts in pAF, consistent with observed increases in the expression of matrix metalloproteinase 9 (REF. 77), collagen 1, and collagen 3, collagen volume fraction, and atrial fibrosis in right atrial tissues of patients with pAF compared with individuals in sinus rhythm[78–80].

Animal models of spontaneous pAF

Currently used animal models have substantial limitations with respect to the extrapolation of findings to human AF, particularly pAF[6,81,82]. One major problem is the paucity of experimental models for the spontaneous initiation and recurrence of AF episodes, which typify pAF. In the majority of models, AF is artificially initiated during EPS[81–84]. Programmed electrical stimulation is employed to induce AF, with premature extrastimuli or burst pacing mimicking arrhythmia induction by premature beats or atrial tachycardias that engage a re-entrant substrate. Although the duration of induced AF and the ease of induction provide valuable information about the arrhythmogenic substrate and the potential mechanisms maintaining AF, the mechanisms of AF initiation in such models at best only partially recapitulate spontaneous AF occurrence in humans. Some models maintain atrial tachycardia or AF over prolonged periods (weeks to months) via high-frequency atrial pacing, to provide mechanistic insights into the arrhythmia-maintaining substrate produced by sustained high atrial rates; however, in such models, AF rarely begins spontaneously in the absence of pacing[81,83–85]. Nevertheless, some animal models do show spontaneous atrial tachyarrhythmia or AF onset and termination, and can provide insights into mechanisms underlying pAF. Most of these involve mice engineered to mimic molecular abnormalities in patients with AF, but some have also been developed in larger species, as summarized in TABLE 1.

To date, 20 defined genetic models with spontaneous atrial ectopy, atrial tachycardia, or AF are described in the literature (TABLE 1). These mouse models fall into three broad categories: those with a predisposition to focal ectopic firing, those with electrophysiological abnormalities that promote re-entry by abnormal atrial repolarization, and those with atrial conduction abnormalities that are likely to produce a re-entry substrate. One common observation is that the majority of these genetic models have cardiomyopathic phenotypes with atrial structural remodelling. Overall, consistency exists between the changes detected in atrial tissue from patients with pAF and findings in genetic models of spontaneous AF (TABLE 1; FIGS 3,4). Focal ectopic firing and cellular triggered activity were observed in three specific mouse models (CREM-IbΔC-X[66,86,87]; *Casq2*$^{-/-}$ (REF. 88); *Dct*$^{-/-}$ (REF. 89)), atrial APD/ERP abnormalities (shortening, prolongation, or increased heterogeneity) were noted in ten defined genetic models (F1759A-Na_v1.5-dTG[90]; *Kcne1*$^{-/-}$ (REF. 91); K_{ir}2.1-TG[92]; *Nup155*$^{+/-}$ (REF. 34); CREM-IbΔC-X[66,86,87]; $G\alpha_q$-TG[93]; TG-junctate-1 (REF. 94); TG-junctin[95]; MHC-TGFcys^{33}ser[96]; *Dct*$^{-/-}$ (REF. 97)), and abnormal atrial conduction (connexin changes, extracellular matrix remodelling including fibrosis) providing a substrate for AF was present in 15 of these mouse lines (ACE 8/8-TG[98]; $G\alpha_q$-TG[93]; AMPK-TG^{N488I} (REF. 99); LKB1-KO[97]; F1759A-Na_v1.5-dTG[90]; D1275N-Na_v1.5 (REF. 100); dnPI3K-DCM[101]; RTEF-1-TG[102]; CREM-IbΔC-X[66,86,87]; TG-junctate-1 (REF. 94); TG-junctin[95]; MHCsTNF-TG[103]; RacET[104]; MHC-TGFcys^{33}ser[96]; *Casq2*$^{-/-}$ (REF. 88)). Some specific differences also exist between observations in genetic models and those obtained in patients with pAF. For instance, RyR2 protein levels are increased in patients with pAF[36,48], whereas spontaneous AF in some mouse lines is associated with reduced RyR2 expression (TG-junctate-1 (REF. 94); TG-junctin[95]). Atrial APD/ERP abbreviation (*Kcne1*$^{-/-}$ (REF. 91); K_{ir}2.1-TG[92]; *Nup155*$^{+/-}$ (REF. 34); MHCsTNF-TG[103]) or prolongation ($G\alpha_q$-TG[93]; F1759A-Na_v1.5-dTG[90]; CREM-IbΔC-X[66,86,87]; TG-junctate-1 (REF. 94); TG-junctin[95]; *Dct*$^{-/-}$ (REF. 89)) are both associated with spontaneous AF in genetic mouse

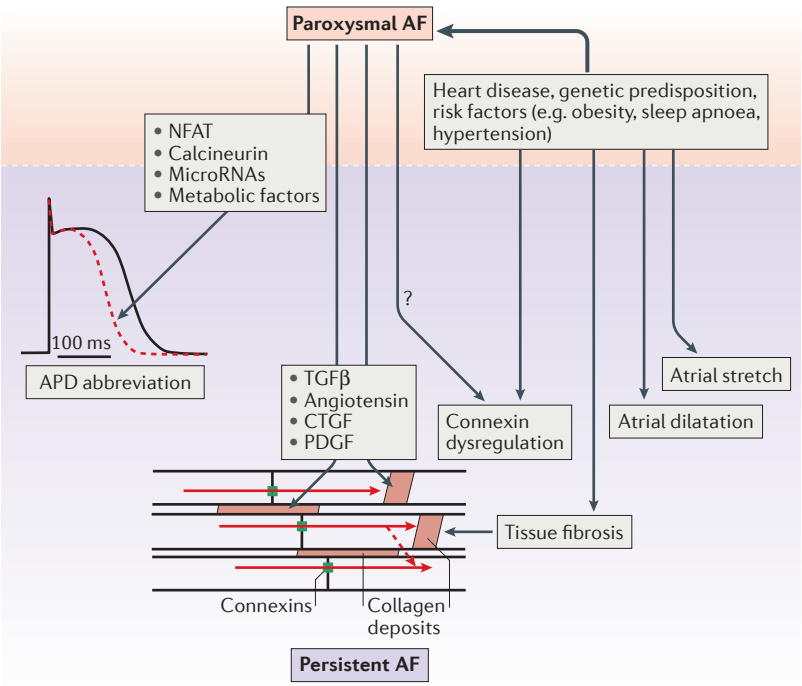

Figure 5 | **Mechanisms of progression from paroxysmal atrial fibrillation (AF) to persistent forms.** Action potential duration (APD) abbreviation resulting from AF-induced ion-current remodelling abbreviates refractoriness and promotes re-entry. Similarly, dysregulation of connexin proteins and the production of atrial fibrosis cause AF-promoting conduction abnormalities. The underlying cardiac conditions that cause the initial occurrence of paroxysmal AF can also lead to fibrosis, atrial stretch, and atrial dilatation that favour re-entrant AF-maintaining mechanisms. CTGF, connective tissue growth factor; NFAT, nuclear factor of activated T-cells; PDGF, platelet-derived growth factor; TGFβ, transforming growth factor-β.

lines, whereas atrial APD/ERP is unchanged in tissue samples from patients with pAF[48,57]. Conversely, whereas specific APD/ERP data are not available, gain-of-function K^+-channel mutations do cause clinical pAF[30–32], and are likely to be associated with APD/ERP abbreviation.

A few nongenetic animal models with spontaneous AF also recapitulate important findings relevant to clinical pAF (TABLE 1). Long-standing arterial hypertension in rats leads to spontaneous AF resulting from autonomic imbalance with relative vagal hyperactivity, atrial hypertrophy, and altered connexin expression[105,106]. Dogs with chronic atrial ischaemia show spontaneous atrial ectopy and atrial tachycardia associated with enhanced triggered activity, together with increased conduction heterogeneity associated with atrial fibrosis[107]. In dogs with congestive heart failure owing to ventricular tachypacing, spontaneous atrial ectopy and AF result from DAD-mediated triggered activity combined with increased atrial fibrosis, altered connexin expression, and enhanced conduction heterogeneity[108–110]; many of the same abnormalities have been detected in tissues from patients with pAF (see above). Dogs and pigs with autonomic remodelling and pharmacological sympathovagal activation also develop spontaneous atrial tachycardia or AF[111–114], consistent with the finding that clinical AF is frequently preceded by sympathetic activation followed by enhanced vagal nerve activity just before AF initiation[26,27].

Animal models provide insights into potential cellular and molecular components of pAF pathophysiology that have not been investigated in atrial tissue from patients with pAF. For instance, hyperpolarization-activated cyclic nucleotide-gated (HCN) channel subunit remodelling in congestive heart failure results in enhanced HCN4 subunit expression, which is likely to lead to abnormal atrial automaticity contributing to spontaneous focal ectopic firing and atrial tachycardia in this model[115] (FIG. 3). In addition, transgenic mouse models with cardiac-specific expression of mutations in the human sodium channel $Na_v1.5$ show increased persistent Na^+ current along with prolonged atrial APD and atrial remodelling (atrial fibrosis, atrial hypertrophy, increased glycogen deposition, atrial conduction disturbances) resulting in spontaneous AF[90,100]. Furthermore, remodelling of atrial fibroblast ion channels (I_{K1}, I_{Kv}, transient receptor potential cation channel subfamily C member 3 [TRPC3] and perhaps transient receptor potential cation channel subfamily M member 7 [TRPM7]) contributes to the differentiation of fibroblasts into collagen-secreting myofibroblasts in dogs with congestive heart failure[116–118]. Whether abnormal HCN channel function and increased persistent Na^+ current in atrial cardiomyocytes and ionic remodelling in atrial fibroblasts contribute to the pathophysiology in patients with pAF is unknown.

Taken together, the data available from animal models provide insights into the cellular and molecular determinants of spontaneous AF episodes, although many outstanding questions and issues remain that require further experimental testing in both animal models and atrial tissue from patients with pAF. In addition, the limitations of animal models must be considered. All animal models are only controlled paradigms mimicking aspects of the human condition. None of them reproduces the complex set of conditions and variables present in patients with AF. Rodent models are limited by differences in action potential properties, and particularly differences in repolarizing currents, compared with those in humans. Even large-animal models such as dogs have differences in detailed repolarizing-current properties compared with humans[119]. Nevertheless, animal models are useful for testing specific hypotheses about basic mechanisms and uncovering mechanistic components for further testing in human studies.

Progression to persistent AF

Persistent AF is associated with therapeutic resistance and complications. The rate of progression from pAF to persistent AF is substantial, and prevention of progression has been advocated as a therapeutic strategy[9]. FIGURE 5 is a schematic representation of some of the mechanisms believed to lead to AF progression. Indirect evidence implicates pAF occurrence and frequency as a causal factor in progression[120], although atrial remodelling owing to underlying heart disease and risk factors certainly also contributes[9,121]. Experimental data provide some limited insights into the mechanisms underlying AF progression. In a seminal paper, Wijffels *et al.* showed that electrically maintained AF in goats progresses from self-terminating episodes (by definition paroxysmal)

to persistent forms over several weeks[122]. Refractoriness shortening occurs rapidly owing to $I_{Ca,L}$ downregulation[123], and certainly contributes to progression by promoting re-entry, but reaches steady-state well before AF becomes persistent[122], indicating that other factors are involved in progression. $I_{Ca,L}$ downregulation results at least in part from cellular adaptation to rate-related Ca^{2+} loading, which activates calcineurin–NFAT signalling to cause transcriptional downregulation[124]. MicroRNA-26 (miR-26) controls the expression of the main I_{K1} subunit KCNJ2 and is also downregulated by calcineurin–NFAT signalling, resulting in I_{K1} upregulation that reduces APD and is likely to stabilize re-entrant rotors[47,125,126]. Other factors that have been implicated include remodelling of cellular ultrastructure[127], connexin changes[128], and epicardial fibrosis that disrupts muscle-bundle continuity[129]. Rapidly-firing atrial cardiomyocytes produce substances that activate fibroblasts, providing a plausible link between pAF episodes and fibrosis development[130]. The involvement of fibrosis has also been implicated in a sheep model of electrically maintained AF, which showed that ion-current remodelling and action potential changes occur rapidly during AF, but left atrial fibrosis has a slower onset that correlates with the development of persistent AF[85]. MiR-26-induced I_{K1} upregulation in cardiac fibroblasts contributes to fibrosis development by enhancing fibroblast-activating Ca^{2+} entry[116]. TRPC3 is also controlled by miR-26, and the upregulation of TRPC3 causes fibroblast activation, contributing to AF progression[118].

Box 1 | **Future research questions**

What makes paroxysmal atrial fibrillation (pAF) episodes self-terminate?
The characteristic feature that differentiates pAF from persistent AF is its self-terminating nature. However, we have very little understanding of why pAF episodes self-terminate. If a pAF episode begins and is maintained for a while, does something change (for example, autonomic tone, atrial wall stress) to cause termination? Or is self-termination simply a statistical function of instantaneous probabilities projected over time? Can the determinants of termination be manipulated to increase the probability that the arrhythmia will stop? The issue of self-termination is central to the identity of pAF, and the mechanisms determining this cardinal distinguishing feature must be better defined in order for our understanding and management options to improve.

Which signalling mechanisms lead to pAF and can they be prevented?
Given that we tend to study pAF after it has manifested, we know very little about the signalling mechanisms leading to its initial occurrence. We need to understand these better in order to interrupt them and prevent pAF onset.

How representative are tissue samples from specific patient populations?
Some of the most interesting and potentially important observations about the molecular mechanisms in pAF come from samples obtained from patients undergoing cardiac surgery. However, because of practical considerations, these studies are always based on a limited number of patients, with specific features and characteristics. Patients with pAF undergoing cardiac surgery are not necessarily representative of the broader pAF population — after all, they must have at least one other serious cardiac condition because surgery is almost never performed for pAF per se. It will be important in the future to test specific observations and notions in patient populations other than the one(s) in which they were initially obtained.

Can specific mechanisms be identified and targeted in patients with pAF?
There are many candidate mechanisms that might underlie pAF and, at present, whether or how we can identify those operative in individual patients is uncertain. Even at the simplest level, can methods be developed to establish the extent to which focal ectopic activity versus re-entry is operative in individual patients, as they might require different therapeutic approaches?

Which experimental mechanisms are clinically important, and can they be targeted therapeutically?
Even a glance, the information shown in FIG. 3 and in FIG. 4 indicates that a host of molecular determinants might participate in governing pAF. We need to know whether they are all clinically relevant, or whether some candidate mechanisms that seem promising in model systems have no role in humans. In the presence of multiple systems governing AF properties and occurrence in individual patients, can we identify the main 'vulnerable targets' and 'check points' to serve as a basis for clinically effective therapies?

What are the roles of underlying heart disease versus AF-induced remodelling in AF progression?
Although pAF often progresses to persistent AF, and mechanisms have been identified experimentally through which pAF can promote the development of a substrate for persistence, how important are these mechanisms? An alternative and plausible hypothesis is that the conditions that led to pAF continue to progress and inexorably lead to AF persistence, with minimal or no contribution from the pAF episodes per se. If this were the case, preventive therapy would need to be directed at the underlying cardiac pathology rather than AF episodes that are simply a manifestation of the common underlying condition(s) rather than the cause of eventual persistence.

Does treating AF risk factors reduce pAF burden and progression?
We now know that AF risk factors lead to atrial remodelling that forms the substrate for AF[1]. It is important to know whether the management of reversible risk factors, such as obesity, obstructive sleep apnoea, cigarette smoking, or hypertension, can lower the pAF burden, provide symptomatic improvement, and prevent progression. Interesting observations have been obtained in obese-population intervention studies[141], but more work is needed in other populations and for specific risk factors. In addition, given that we might now be able noninvasively to detect and quantify aspects of the substrate such as fibrosis[142] and conduction abnormalities[143], it will be important to determine how risk-factor intervention affects the substrate directly.

What basic mechanisms control thromboembolism in pAF?
The most important complication in AF is thromboembolism, but we know very little about its basic mechanistic determinants. Atrial appendage stasis is clearly very important, but changes in endocardial thromboresistance and coagulation elements are also thought to have a role[144]. There are many puzzling aspects of thromboembolism occurrence in pAF, such as a lack of temporal correlation with pAF episodes and, at least until now, a lack of clear prevention with rhythm control. A better understanding is needed to optimize stroke prevention in patients with pAF.

Is persistent AF always less desirable than pAF?
It is generally assumed that progression from pAF to persistent AF is undesirable and should be prevented, but is this always the case? Some patients are highly symptomatic with pAF, and perhaps because of systemic adaptation less so in persistent AF. Can 'progression' sometimes be desirable? If so, can we identify patients in whom AF progression is a desirable rather than adverse outcome and manage them accordingly?

Are we missing fundamental AF mechanisms?
Although our knowledge of the detailed mechanistic basis of AF has advanced enormously, our basic concepts remain remarkably similar to ideas first advanced a century ago[145]. Is this because of the prescience of the initial ideas, or is it because our ideas are stuck in orthodox ways of thinking? The latter possibility should at least be seriously considered in order to avoid overlooking potentially important new concepts.

Although experimental work in models with electrically maintained AF can provide insights into how AF transitions from self-terminating to persistent forms, they cannot be directly translated to pAF, for which the episodes are separated by periods of sinus rhythm during which recovery from remodelling can occur. In goats, three consecutive 5-day periods of AF separated by 48 h in sinus rhythm had no cumulative effect[131]. Conversely, 4-week periods of AF separated by enough time in sinus rhythm to allow full recovery of refractoriness (6 ± 2 days) resulted in a progressive reduction in the AF time needed to induce long-lasting (>24 h) AF and a progressive increase in the induction rate of AF by premature extrastimuli[132]. Another study showed that daily 3-h AF episodes produce structural, ion-current, and refractory-period remodelling over a 4-week period[133]. In a mouse model that progresses spontaneously from pAF to persistent episodes, genetically-engineered prevention of SR Ca^{2+} leak (by blocking RyR2 phosphorylation via CaMKII) prevents progression, implicating Ca^{2+} signalling and CaMKII in AF progression[66]. Taken together, the results indicate that repeated pAF leads to electrophysiological changes that can promote AF persistence, but much more work needs to be done to understand the underlying mechanisms and clinical relevance.

Clinical implications

Little attention has been paid specifically to pAF in the experimental literature and, even in the clinical literature, few papers have dealt with its mechanisms specifically. AF very commonly begins as pAF, and then often progresses to the persistent and sometimes long-standing persistent form. The time at which to target AF is, therefore, most likely to be at its earlier and less therapy-resistant paroxysmal stage. Accordingly, early and vigorous therapy has been suggested to prevent AF progression and complications[9]. A better understanding of pAF mechanisms should help to develop and target new therapies for AF, and would also help in better understanding the natural history of AF, appreciating links to associated diseases and conditions, and developing mechanism-based classifications of AF to guide patient-specific treatment[134].

The pathophysiological notions discussed in this paper have potential therapeutic implications. First, they point the way to novel treatment targets. The evidence for a central contribution of RyR2 leak and SR dysfunction in pAF suggest directions for new drugs that suppress focal activity. Approaches could be small-molecule medications, but could also include biologicals with more specific actions[135]. Better understanding of the substrate for re-entry might allow for improved methods to prevent the substrate from developing and even to cause substrate regression. Mechanistic concepts might also lead to improved nonpharmacological approaches such as ablation therapy[136]. One definite example was the identification of the mechanisms by which adenosine unmasks PVs with 'dormant conduction' at risk of reconnection after an ablation procedure[137]. A subsequent randomized trial showed that routine use of intravenous adenosine to identify dormant veins led to improved outcomes with PV-isolation procedures for pAF[17]. An improved appreciation of the control of pAF onset by the autonomic nervous system might lead to new methods to analyse the dynamic substrate and prevent arrhythmias[138]. Another application might be optimized selection of appropriate study populations for new antiarrhythmic drug development. Patients with a high burden of self-terminating pAF are likely to have a predominance of focal ectopic mechanisms. Therefore, such patients are probably not the best group in which to study new antiarrhythmic drugs designed to prevent re-entrant AF; negative outcomes in such trials[139] are potentially both misleading and expensive. Specific targets and considerations regarding translation from studies of AF mechanisms to clinical therapeutics have been reviewed in detail recently[140]. BOX 1 lists ten unresolved questions in pAF pathophysiology that need to be addressed in future work.

Conclusions

We have learned an enormous amount about the basic mechanisms of AF, but only a limited part of it relates directly to pAF. Given that most AF begins in a paroxysmal form, we need to learn much more about the specific pathophysiological and molecular determinants of pAF, and how these can be applied to improve clinical management.

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Both authors researched data for the article, discussed its content, wrote the manuscript, and reviewed/edited it before submission.

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