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Atrial fibrillation (AF) is an extremely common cardiac rhythm disorder that causes substantial morbidity and contributes to mortality. The mechanisms underlying AF are complex, involving both increased spontaneous ectopic firing of atrial cells and impulse reentry through atrial tissue. Over the past ten years, there has been enormous progress in understanding the underlying molecular pathobiology. This article reviews the basic mechanisms and molecular processes causing AF. We discuss the ways in which cardiac disease states, extracardiac factors, and abnormal genetic control lead to the arrhythmia. We conclude with a discussion of the potential therapeutic implications that might arise from an improved mechanistic understanding.

Recent advances in the molecular pathophysiology of atrial fibrillation

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Atrial fibrillation (AF) is an extremely common cardiac rhythm disorder that causes substantial morbidity and contributes to mortality. The mechanisms underlying AF are complex, involving both increased spontaneous ectopic firing of atrial cells and impulse reentry through atrial tissue. Over the past ten years, there has been enormous progress in understanding the underlying molecular pathobiology. This article reviews the basic mechanisms and molecular processes causing AF. We discuss the ways in which cardiac disease states, extracardiac factors, and abnormal genetic control lead to the arrhythmia. We conclude with a discussion of the potential therapeutic implications that might arise from an improved mechanistic understanding.

Introduction

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia, and its prevalence is increasing with aging of the population (1). Normal cardiac rhythm shows regular rhythm initiation in the sinoatrial (SA) node, followed by atrial and then ventricular activation (Figure 1A). Abnormal spontaneous firing (ectopic activity) from sources other than the SA node is absent. AF is reflected in the ECG recording by the replacement of regular P-waves by an undulating baseline (reflecting continuous, rapid, spatially heterogeneous atrial activation) and irregular ventricular QRS complexes (Figure 1B). Uncoordinated atrial activity prevents effective atrial contraction, leading to clot formation in the blind pouch atrial appendage. Irregular and inappropriately rapid ventricular activity interferes with cardiac contractile function.

AF contributes significantly to population morbidity and mortality, and presently available therapeutic approaches have major limitations, including limited efficacy and potentially serious side effects such as malignant ventricular arrhythmia induction (2). An improved understanding of the mechanisms underlying AF is needed for the development of novel therapeutic approaches (3). A detailed review nine years ago highlighted progress in understanding AF pathophysiology and outlined important unresolved issues (4); since then, knowledge has greatly increased. The purpose of the present article is to summarize these recent findings, particularly in the area of molecular pathophysiology.

Pathophysiological mechanisms

Pathophysiological mechanisms and relation to AF forms

To understand the molecular mechanisms underlying AF, it is necessary to place them in a pathophysiological context. Because of their importance, these mechanisms will be discussed briefly here (for more detailed treatments, see refs. 4, 5).

Focal ectopic activity. The mechanisms believed to produce ectopic activity from atrial foci are illustrated in Figure 2. Normal atrial cells ("Normal atrial action potentials" in Figure 2A) display typical voltage changes over time. They start at a negative intracellular membrane potential (the resting potential), become very positive when fired (depolarized) during a period called phase 0, then go through a series of repolarizing steps to get back to the resting potential, at which they remain until the next action potential. Automatic activity occurs when an increase in time-dependent depolarizing inward currents carried by Na^+ or Ca^{2+} (making the cell interior more positive) or a decrease in repolarizing outward currents carried by K^+ (which keep the cell interior negative) causes progressive time-dependent cell depolarization. When threshold potential is reached, the cell fires, producing automatic activity. If automatic firing occurs before the next normal (sinus) beat, an ectopic atrial activation results.

Delayed afterdepolarizations (DADs; Figure 2B) constitute the most important mechanism of focal atrial arrhythmias. They result from abnormal diastolic leak of Ca^{2+} from the main cardiomyocyte Ca^{2+} storage organelle, the sarcoplasmic reticulum (SR). The principal Ca^{2+}-handling mechanisms governing DAD-related firing (triggered activity) are shown in Figure 2D. Ca^{2+} enters cardiomyocytes through voltage-dependent Ca^{2+} channels during the action potential plateau, triggering Ca^{2+} release from the SR via Ca^{2+} release channels known as ryanodine receptors (RyRs; RyR2 is the cardiac form). This systolic Ca^{2+} release is responsible for cardiac contraction. Following action potential repolarization, diastolic cardiac relaxation occurs when Ca^{2+} is removed from the cytosol back into the SR by a Ca^{2+} uptake pump, the SR Ca^{2+} ATPase (SERCA). DADs result from abnormal diastolic Ca^{2+} leak through RyR2 from the SR to the cytoplasm (6). Excess diastolic Ca^{2+} is handled by the cell membrane Na^+,Ca^{2+}-exchanger (NCX), which transports three Na^+ ions into the cell per single Ca^{2+} ion extruded, creating a net depolarizing current (called transient inward current, or I_{ti}) that produces DADs. DADs that are large enough to reach threshold cause ectopic firing. Repetitive DADs cause focal atrial tachycardias (tachycardia is a heart rhythm >100 bpm). RyR2s are Ca^{2+} sensitive, and RyR2 leak results from SR Ca^{2+} overload or intrinsic RyR2 dysfunction. RyR2 function is modulated by channel phosphorylation: hyperphosphorylation makes RyRs leaky and arrhythmogenic (7). Loss or dysfunction of calse-

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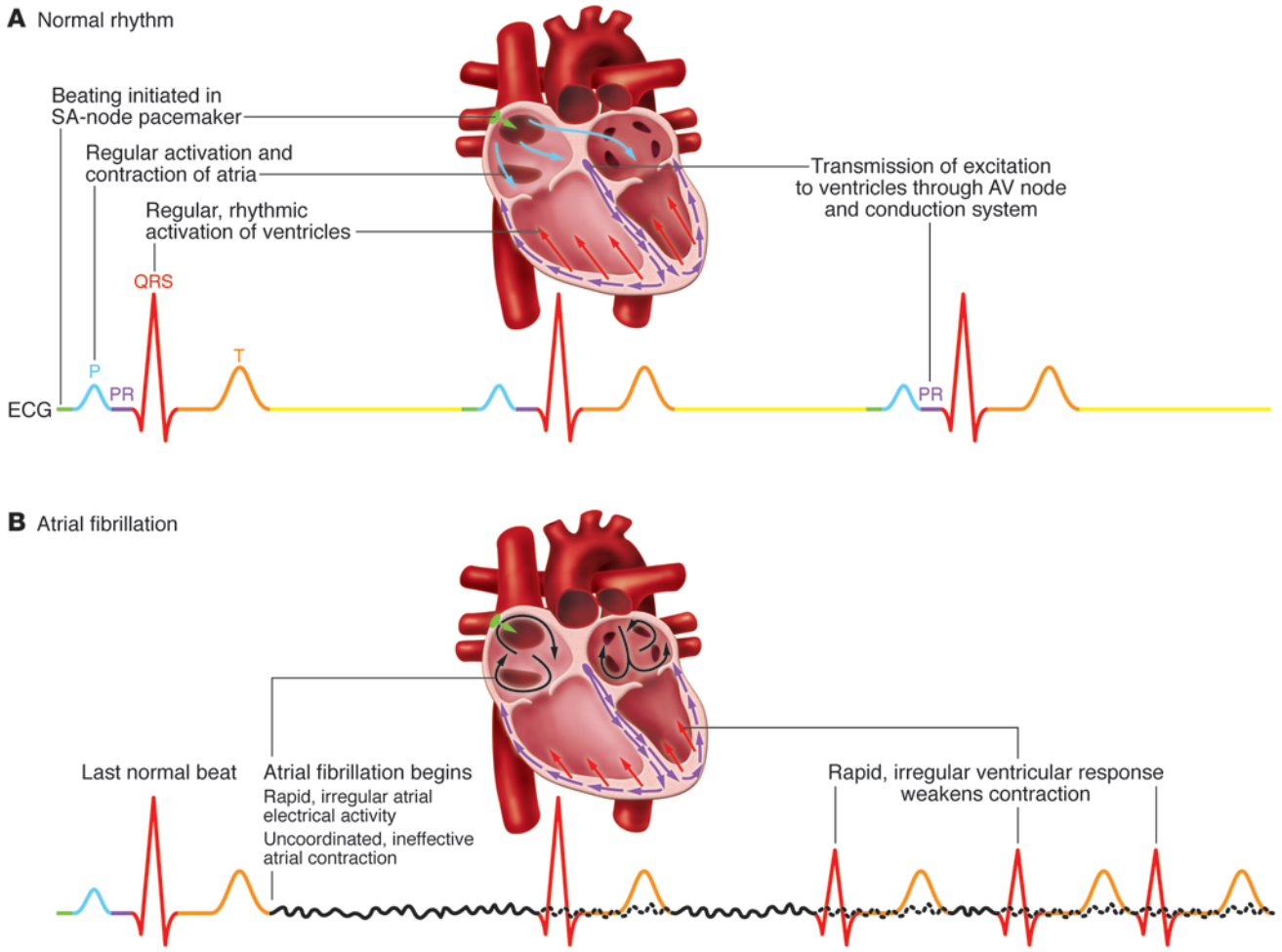

Figure 1
ECG recordings of sinus rhythm and AF. (**A**) Bottom: A normal ECG recording showing sinus rhythm. Top: Schematics of major events in one cardiac activation cycle: rhythm is initiated by the SA node pacemaker, resulting in atrial activation, followed by atrioventricular conduction via the AV node and His-Purkinje conducting system, leading to ventricular activation. (**B**) ECG showing onset of AF after one regular sinus beat. Atrial activation is now rapid and irregular, producing an undulating baseline that is visible when not obscured by larger QRS and T waves (continuous atrial activity during this phase is represented by dotted lines). During AF, rapid and uncoordinated atrial activity leads to ineffective atrial contraction. Ventricular activations (QRS complexes) now driven by the fibrillating atria occur rapidly and irregularly, weakening cardiac contraction efficiency and causing clinical symptoms.

questrin (CSQ), the main SR Ca^{2+}-binding protein, exposes RyRs to excess free SR Ca^{2+} (7).

When action potential duration (APD) is excessively prolonged, cell membrane Ca^{2+} currents recover from inactivation and allow Ca^{2+} to move inward, causing early afterdepolarizations (EADs; Figure 2C). APD prolongation is spatially variable (4). Cells that generate EADs adjacent to more normally repolarizing cells raise the latter to threshold, causing them to fire and to initiate focal activity (4).

It must be emphasized that the mechanisms depicted in Figure 2 are based on concepts developed previously in ventricular tissue, and that the evidence for their involvement in atrial arrhythmias, particularly clinical AF, remains fragmentary. Particularly limiting is the paucity of reliable clinically relevant animal models of spontaneous AF occurrence.

Reentry. Reentry requires appropriate tissue properties, a vulnerable "substrate." Reentry substrates can be caused by altered electrical properties or by fixed structural changes. Cardiac tissue exhibits a discrete refractory period (inexcitable interval following the last firing, governed by APD). Reentry initiation usually requires a premature ectopic beat that acts as a trigger. Figure 3, A–C, shows a premature beat arising at a branch point (labeled "i"). The resulting impulse conducts through the pathway leading to recording point ii, which is no longer refractory, but blocks in the pathway leading to recording point iii because of its longer refractory period. The premature impulse arrives at the distal end of previously refractory site iii and attempts to reenter. Under normal conditions without a reentry substrate (Figure 3A), the conduction time from point i around the circuit through point ii and back through point iii is shorter than the refractory period, and the impulse cannot reenter. When APD is decreased, reducing the refractory period sufficiently (Figure 3B), excitability is recaptured earlier and the reentering impulse can now sustain itself throughout the circuit. Slowed conduction can similarly allow the impulse to reenter (without APD abbreviation), because the more slowly

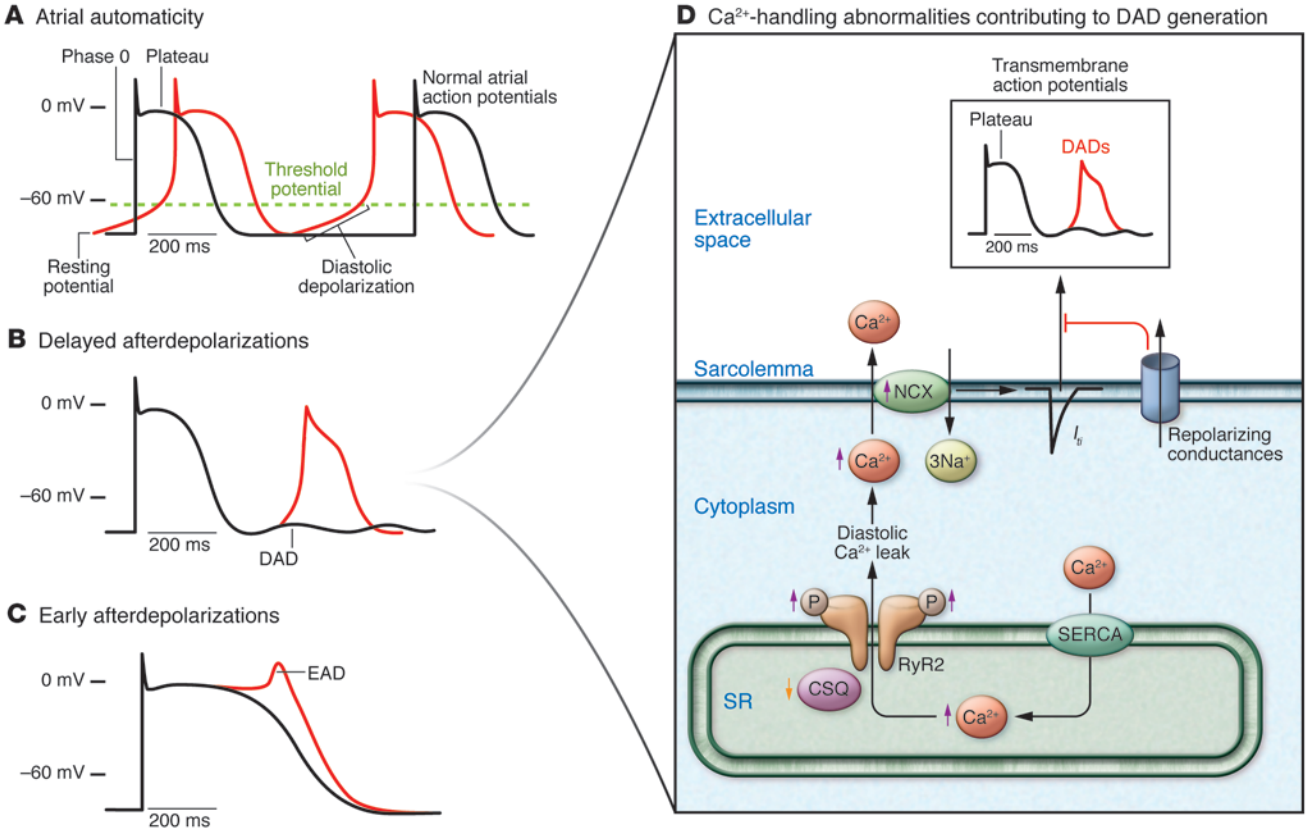

Figure 2
Cellular mechanisms underlying focal ectopic activity. (**A**) The normal atrial action potential (transmembrane potential as a function of time, in black) has a stable resting value close to –80 mV. Cell firing causes rapid depolarization (phase 0) to a positive value. Following initial repolarization, there is a flat (plateau) phase and then repolarization back to the resting potential. Normal atrial cells remain at the resting potential until they are fired through the SA node pacemaking system. Abnormal atrial automaticity results from spontaneous diastolic depolarization to a threshold value for activation. (**B** and **C**) Afterdepolarizations: abnormal membrane depolarizations after completion of the AP. DADs occur after full repolarization (**B**); EADs precede full repolarization (**C**). (**D**) Fundamental mechanisms leading to DADs, the most important source of ectopic activity in AF. DADs result from spontaneous diastolic SR Ca^{2+} releases through channels called RyR2s. RyR2s are sensitive to intra-SR free Ca^{2+} concentration. Abnormal diastolic RyR2 Ca^{2+} releases can result from excess SR intraluminal Ca^{2+} (pumped into the SR by SERCA) or reduced SR Ca^{2+} binding by the principal SR Ca^{2+} buffer, calsequestrin (CSQ). RyR2 hyperphosphorylation increases sensitivity to SR Ca^{2+}, causing abnormal RyR2 Ca^{2+} release events. Diastolic RyR2 Ca^{2+} release increases cytosolic Ca^{2+}, which has to be removed by the NCX. NCX moves three Na^+ ions into the cell in exchange for each Ca^{2+} ion moved out, creating an inward movement of positive charges that produces a depolarizing I_{ti}. Repolarizing conductances oppose I_{ti}, protecting against excessive diastolic membrane voltage oscillations.

conducting impulse leaves additional time for refractoriness to dissipate (Figure 3C). Figure 3D illustrates the development of a fixed structural reentry substrate. A combination of atrial dilation and fibrosis creates longer potential conduction pathways for reentry, slows conduction, and imposes conduction barriers that favor the initiation and maintenance of multiple irregular reentry circuits that sustain AF (5).

Relation of basic mechanisms to clinical forms

Figure 4A shows how basic mechanisms relate to the pathophysiology of AF. Ectopic activity can be transient, manifesting as isolated ectopic beats, or sustained, causing tachycardia. Any source of sustained rapid atrial tachycardia, whether an ectopic focus or regularly discharging reentry circuit, is called a driver. Drivers that discharge rapidly and regularly can cause irregular activity characteristic of AF if the emanating propagation waves break up in functionally heterogeneous atrial tissue, leading to fibrillatory conduction (5, 8). AF-related reentry occurs in two general forms: (a) single-circuit reentry, involving one primary reentry circuit driver; and (b) multiple-circuit reentry, involving multiple simultaneous dyssynchronous reentry circuits. The very rapid activation caused by AF produces atrial tachycardia remodeling (ATR) of atrial electrical properties, promoting functional reentry (4). ATR effects vary in different atrial regions, causing spatial heterogeneity that promotes multiple-circuit reentry (4).

Clinical AF can be paroxysmal (self-terminating), persistent (requiring medical intervention to terminate), or permanent (Figure 4B). Focal drivers, particularly ectopic sites in cardiomyocyte sleeves around the pulmonary veins (9), produce paroxysmal AF forms. Functional reentry substrates cause persistent AF that can be terminated, restoring normal rhythm. AF becomes permanent as the substrate becomes fixed and irreversible because of structural remodeling (10). AF itself induces remodeling (4, 11): ATR, which promotes functional reentry, as well as structural remodeling.

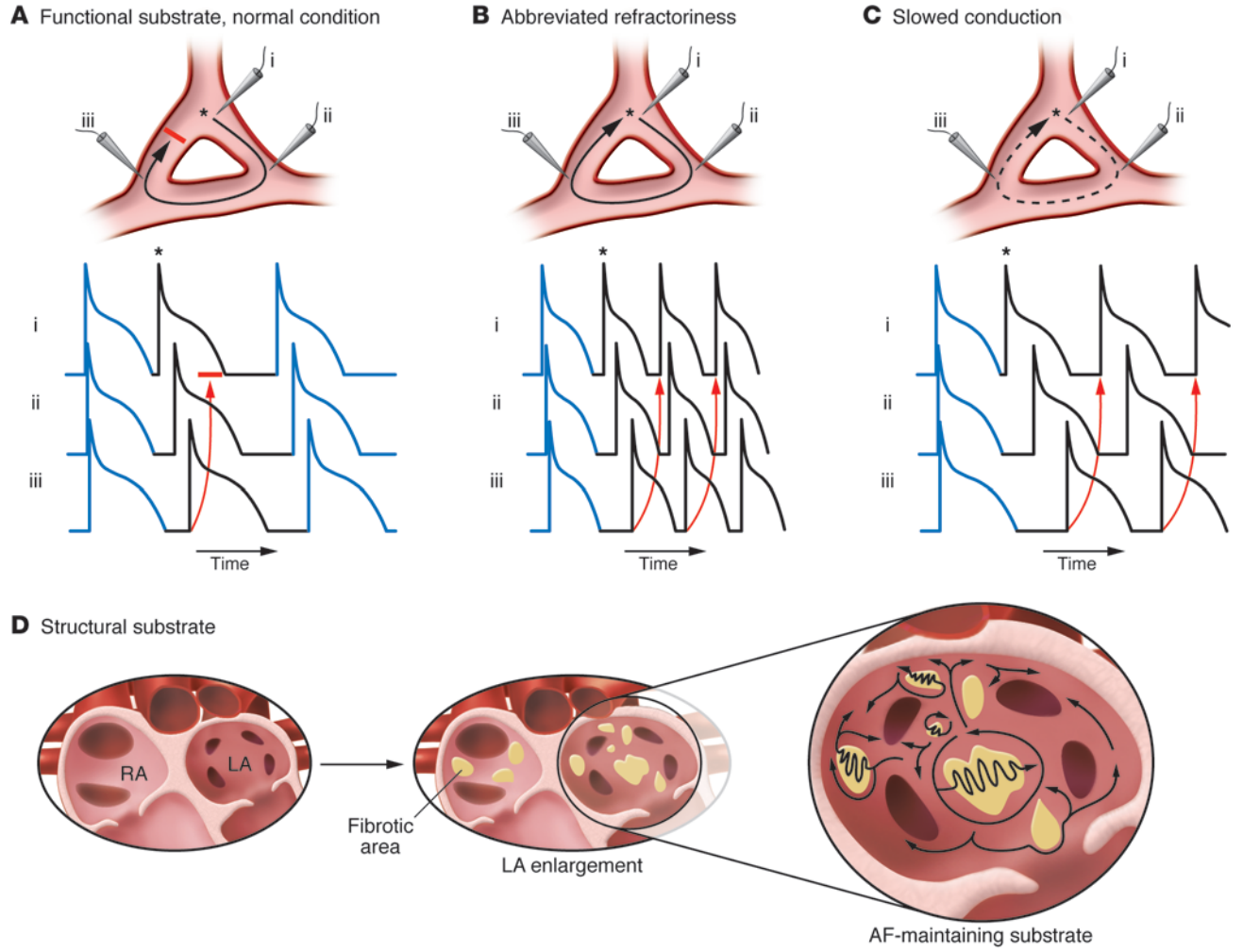

Figure 3
Factors promoting reentry. Reentry occurs via interactions between interconnected zones of tissue, initiated by a premature beat (*). i, ii, and iii indicate microelectrode recordings in three zones of a potential reentry circuit. (**A**) Normal atrial tissue is unlikely to maintain reentry. Reentry maintenance can result from either a shortened refractory period (**B**) or slowed conduction (**C**). (**D**) A variety of cardiac conditions cause structural reentry substrates characterized by atrial enlargement and fibrosis. LA, left atrium; RA, right atrium.

Etiological contributors

A variety of etiological factors contribute to AF occurrence (Figure 5). In most patients, AF results from interactions among multiple factors operating simultaneously.

Over 70% of AF cases have associated heart disease (12). Aging is a major risk factor, largely via structural remodeling (3, 11). Congestive heart failure (CHF), hypertension, valvular heart disease, and coronary artery disease (CAD) are common contributors (11). Less common predisposing conditions include peri- or myocarditis, atrial myxomas, and hypertrophic cardiomyopathy. Extracardiac conditions also promote AF occurrence. Sufficiently powered studies suggest that heavy alcohol consumption promotes AF (13). Hyperthyroidism is a well-recognized contributor (14), and the roles of sleep apnea and obesity are increasingly recognized (15). Autonomic tone is a well-established factor (16). Ten years ago, genetic determinants of AF were largely unknown (4), but there has since been an explosion of information (17, 18). Many disease-causing mutations have been identified and their pathophysiology analyzed (17). While new insights have been provided, these findings raise as many questions as they answer (17). The recent rapid development of large-scale genome-wide association studies (GWASs) has provided exciting leads about genetic predisposition to AF, while raising challenging pathophysiological issues (18).

We will now consider how each of the three principal categories of etiological contributors in Figure 5 (heart disease, extracardiac factors, and genetic determinants) act through the pathophysiological mechanisms in Figures 2–4 (focal ectopic activity, functional reentry substrates, and fixed reentry substrates).

Mechanisms underlying AF-promoting ectopic activity

Heart disease–related ectopic activity

Atrial tissue samples from AF patients provide insights into pathophysiology caused by AF and/or underlying heart disease (19). AF atria show abnormal SR Ca^{2+} handling (20–23), which causes spontaneous diastolic SR Ca^{2+} release events (20, 22, 23). SR Ca^{2+}

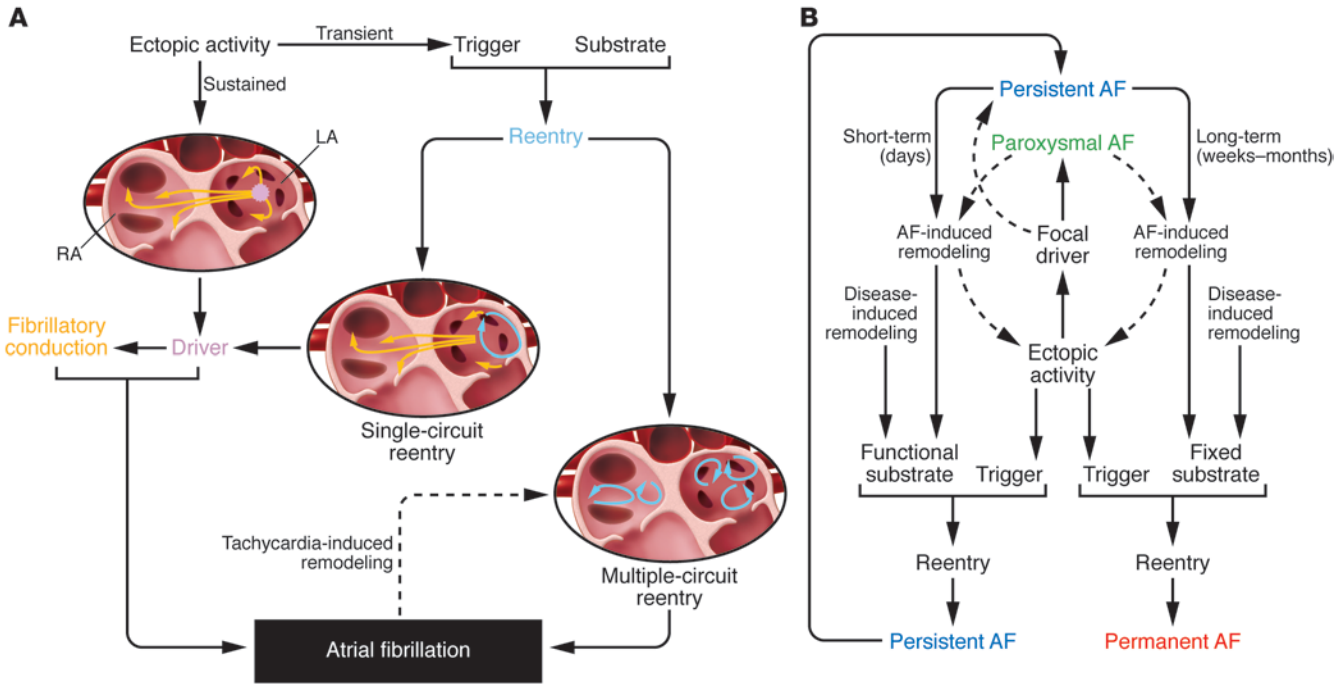

Figure 4
Tissue mechanisms leading to AF and clinical forms. (**A**) Ectopic activity can act as a driver maintaining AF or as a trigger on a vulnerable substrate resulting in reentry (single- or multiple-circuit). Local driver mechanisms (ectopic or single-circuit reentrant) produce irregular fibrillatory activity via fibrillatory conduction. Rapid atrial activity (tachycardia) causes atrial remodeling, promoting multiple-circuit reentry. (**B**) Clinical AF can manifest as paroxysmal AF (self-terminating), persistent AF (requires drug therapy or electrical cardioversion to terminate), and permanent AF (non-terminating). Focal ectopic drivers are principally associated with paroxysmal forms, functional reentrant substrates with persistent AF, and increasingly fixed substrates with permanent forms.

load is not increased (20, 23), suggesting that spontaneous Ca^{2+} releases occur because of altered RyR2 function.

The detailed molecular pathobiology of AF-related DADs is summarized in Figure 6. RyR2 phosphorylation by PKA at Ser2808 (21) and by Ca^{2+}/calmodulin-dependent kinase II (CaMKII) at Ser2814 is increased in AF (23–25). CaMKII activity is normally autoinhibited. Ca^{2+}-calmodulin binding removes autoinhibition, activating CaMKII and causing autophosphorylation that makes CaMKII Ca^{2+}-independent (23). Similar activation may result from CaMKII oxidation. Changes in PKA and CaMKII RyR2 phosphorylation state may result not only from changed kinase activity, but also from alterations in phosphatases (6). RyR2 phosphorylation increases its Ca^{2+} sensitivity, enhancing channel open probability (24). Mice lacking RyR2-inhibitory FK506-binding protein 12.6 or with gain-of-function RyR2 mutations exhibit increased susceptibility to AF, along with increased atrial cell SR Ca^{2+} leak and triggered activity (24, 26). Angiotensin promotes AF, perhaps via CaMKII oxidation and enhanced CaMKII phosphorylation of RyR2 (27), in addition to its well-recognized effect of promoting structural remodeling (10). RyR2 dysfunction can also be induced by Ca^{2+} overload resulting from phospholamban hyperphosphorylation, which removes phospholamban inhibition of SERCA and enhances SR Ca^{2+} uptake. Phospholamban hyperphosphorylation can result from enhanced PKA and/or CaMKII activity or from decreased phosphatase function due to increased activity of a phosphatase-inhibitory protein, I-1, caused by I-1 hyperphosphorylation (6, 28).

Increased NCX expression and/or function are commonly observed in AF (23, 28, 29), suggesting that I_{ti} resulting from given amounts of diastolic SR Ca^{2+} leak may be amplified, enhancing the risk of DADs/triggered activity (6). Cardiac inositol 1,4,5-trisphosphate (IP_3) receptors (IP_3R2) act as Ca^{2+}-transporting pathways that facilitate arrhythmogenic SR Ca^{2+} leak (30). IP_3R2 expression is increased by ATR (31). IP_3R2-coupled amplification of atrial SR Ca^{2+} release events and related arrhythmogenesis (32) may thus contribute to AF-related ectopic activity.

AF is very common in CHF patients, with a prevalence varying from approximately 10% in patients in New York Heart Association (NYHA) heart failure classes II–III to approximately 50% in NYHA class IV (33). Focal drivers and triggered activity contribute to CHF-related AF (34, 35). CHF increases atrial SR Ca^{2+} load and reduces CSQ expression, thereby promoting spontaneous SR Ca^{2+} release (36). Mice overexpressing tumor necrosis factor develop CHF along with abnormal atrial cell diastolic Ca^{2+} release upon Ca^{2+} loading (37).

CAD increases AF risk approximately 3.5-fold (38). Atrial ischemia promotes AF (39, 40), in part by causing spontaneous SR Ca^{2+} release events and increasing NCX function, leading to triggered activity and atrial ectopy (39).

Extracardiac factors contributing to ectopic activity

Adrenergic-dependent RyR2 phosphorylation promotes spontaneous SR Ca^{2+} leak (41). Clinically relevant conditions that cause abnormal DAD-promoting Ca^{2+} handling may require adrenergic stimulation to elicit Ca^{2+} sparks and triggered arrhythmias (39).

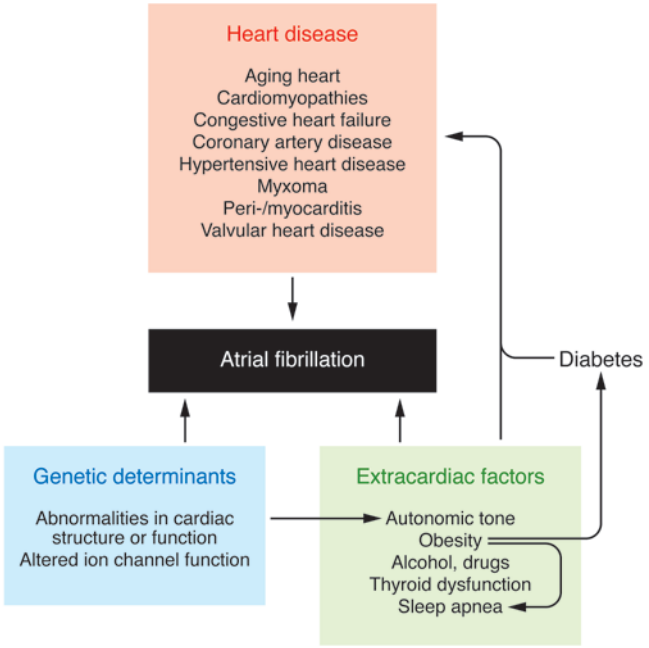

Figure 5
Schematic overview of factors governing AF occurrence.

Autonomic hyperinnervation causes spontaneous paroxysmal AF in dogs (42), with sympathovagal nerve discharge immediately preceding AF paroxysms (43). Vagal activation may promote spontaneous arrhythmogenesis by reducing APD, allowing adrenergically induced afterdepolarizations to induce ectopic activity in susceptible regions such as pulmonary veins (44).

Genetic contributors to ectopic activity

Genetic factors fall into two broad groups: rare genetic variants with strong effects and a clear phenotype (single-gene mutations) and common genetic variants with weaker effects and a less overt phenotype (single nucleotide polymorphisms [SNPs]). Figure 7 provides a broad overview of the mutations/SNPs predisposing to AF and their potential pathophysiological mechanisms. Gene variants believed to cause ectopic activity are indicated in Figure 7A. A mutation in the gene encoding the adapter protein ankyrin-B (long-QT syndrome-4 [LQT4]), which impairs targeting of multiple proteins to the cell membrane, alters Ca^{2+} handling, and leads to DADs/triggered activity (45). A predicted loss-of-function SNP in the gene encoding the SERCA-inhibitory protein sarcolipin is also associated with AF (46).

The first potential genetic cause of AF linked to an EAD mechanism was a loss-of-function SNP in *KCNE1*, which encodes the slow delayed-rectifier K^+ channel (I_{Ks}) β-subunit minK involved in LQT5 (47). The classical EAD-promoting congenital LQTS mutations also predispose to AF (48, 49). A single-gene mutation of the *KCNA5* gene encoding the Kv1.5 α-subunit of the ultrarapid delayed-rectifier I_{Kur} channel identified in an individual with idiopathic AF promotes EADs under adrenergic stimulation (50). Other gene variants in *KNCA5* suggest a role for tyrosine kinases in AF (51). Recent work in a genetic LQTS mouse model (52) directly implicated EAD mechanisms in AF (53).

Recently, two gain-of-function Na^+ channel (*SCN5A*) mutations associated with AF, but with no evidence for EAD-promoting APD prolongation, have been reported (54, 55). These mutations may favor AF initiation by increasing Na^+ channel availability and thereby promoting ectopic activity (54).

Genetic loci on chromosomes 4q25, 16q22, and 1q21 have been associated with AF by GWAS. The first locus on chromosome 4q25 (56) has since been replicated in two additional European cohorts (18). The closest gene is the pair-like homeodomain-2 gene (*PITX2*), which encodes a transcription factor that is crucial for cardiac development and pulmonary vein formation (57, 58). This gene variant may therefore be implicated in pulmonary vein ectopic sources of AF. An AF-associated SNP of the *KCNN3* gene on chromosome 1q21 affects a Ca^{2+}-activated K^+ channel (59). Based on prior work, this mutation may act by abbreviating APD in pulmonary veins, promoting microreentry (60), or cause ectopic activity via EAD mechanisms (61).

Molecular mechanisms underlying functional substrates for AF-maintaining reentry

Heart disease–related functional reentry

AF-induced APD shortening. AF (62), and indeed all very rapid atrial tachyarrhythmias (63), promote AF initiation and maintenance via ATR. A major AF-promoting component of ATR is refractory period reduction due to APD abbreviation (Figure 3B). Decreased depolarizing L-type Ca^{2+} current ($I_{Ca,L}$), along with increased repolarizing inward-rectifier K^+ currents, background (I_{K1}), and constitutively active acetylcholine-dependent current ($I_{K,ACbc}$), underlie ATR-induced APD shortening (64–71). The molecular basis of ATR-induced $I_{Ca,L}$ reduction is complex (Figure 8A). Rapid atrial activation causes Ca^{2+} loading, which activates the Ca^{2+}/calmodulin/calcineurin/NFAT system, causing transcriptional downregulation of the Cav1.2 α-subunit (72, 73). Other potential contributors include downregulation of accessory β_1-, β_{2a}-, β_{2b}-, β_3-, and $\alpha_2\delta_2$-subunits (19, 66, 74, 75), Ca^{2+} channel dephosphorylation due to type 1 (PP1) and type 2A (PP2A) serine/threonine protein phosphatase activation (28, 66), and enhanced Cav1.2 α-subunit S-nitrosylation (76). MicroRNAs (miRNAs) appear to play a major role in AF (77). Recent work implicates increased miR-328 in ATR-induced $I_{Ca,L}$ downregulation (78). Finally, impaired Cav1.2 protein trafficking induced by a zinc-binding protein (ZnT-1) may contribute to $I_{Ca,L}$ reduction (79).

Inward-rectifier K^+ currents play an important role in AF maintenance, by both reducing APD and accelerating arrhythmia-maintaining rotors by hyperpolarizing atrial cells, thereby removing voltage-dependent I_{Na} inactivation (5, 80). Figure 8B shows mechanisms underlying AF-related changes in I_{K1} and $I_{K,ACbc}$. I_{K1} increases because of increased expression of the underlying Kir2.1 subunit (19, 67–71, 81, 82), likely due to reduced levels of Kir2.1-inhibitory miRNAs, particularly miR-1, miR-26, and miR-101 (82, 83). Stronger I_{K1} dephosphorylation (and thus activation) due to increased PP1 and PP2A activity (28, 66, 84) might also contribute.

Muscarinic cholinergic receptor–mediated $I_{K,ACh}$ activation is reduced in AF (69, 70, 85). Loss of receptor-mediated $I_{K,ACh}$ channel control is associated with increased agonist-independent ("constitutive") $I_{K,ACbc}$ (69, 71, 79, 85). Increased $I_{K,ACbc}$ is due to enhanced single $I_{K,ACh}$ channel opening frequency, without changes in single-channel conductance, density, or voltage dependence (85). Enhanced open probability is related to altered PKC isoform balance, with increased $I_{K,ACh}$-stimulatory novel isoform function and reduced inhibitory classical isoform influence (86). Blockade of $I_{K,ACbc}$ suppresses ATR-induced APD abbreviation and AF promotion (70).

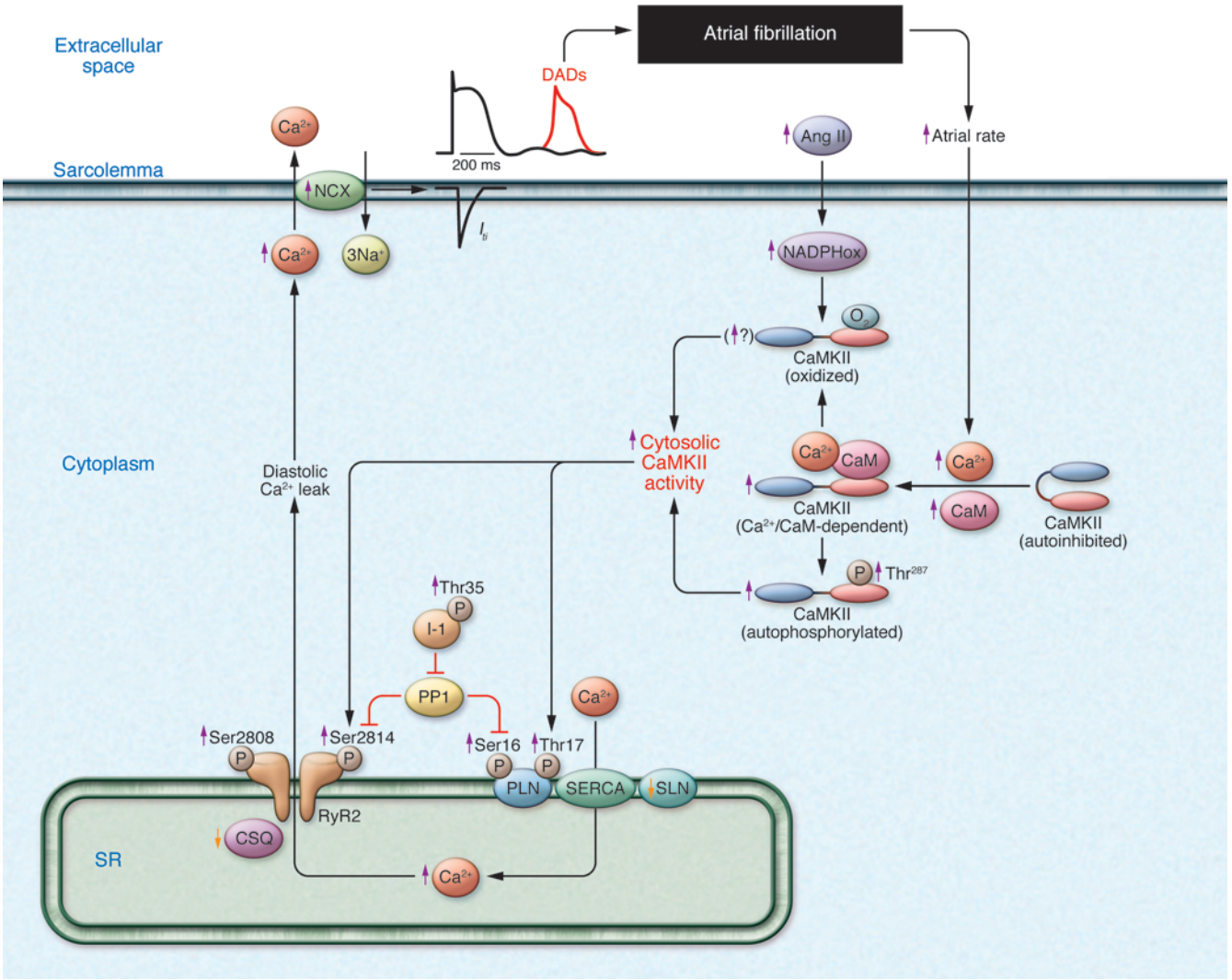

Figure 6
Factors promoting AF by inducing SR diastolic Ca^{2+} leak. RyR dysfunction may result from hyperphosphorylation or exposure to excess intraluminal Ca^{2+}. SR Ca^{2+} overload can result from phospholamban hyperphosphorylation or reduced sarcolipin (SLN) expression, which disinhibit SERCA and enhance SR Ca^{2+} uptake. Increased cellular Ca^{2+} entry due to high atrial rate facilitates Ca^{2+}/calmodulin (CaM) binding to the regulatory domain of CaMKII, resulting in disinhibition of the catalytic subunit. After initial activation of the holoenzyme by Ca^{2+}/CaM, oxidation at Met281/282 or phosphorylation at Thr287 blocks reassociation of the catalytic domain, yielding persistent CaMKII activity. PP1 suppression by enhanced SR compartment inhibitor–1 (I-1 activity) contributes to increased phospholamban (PLN) and RyR phosphorylation. NADPHox, oxidized NADPH.

AF-associated atrial conduction abnormalities. Conduction slowing promotes reentry (Figure 3C). Gap junctions are crucial for cell-to-cell coupling and conduction; however, information in the literature about gap-junctional remodeling in AF is inconsistent (19, 87). Connexin alterations likely vary with AF duration, underlying pathology, and species (88). Spatially heterogeneous connexin-40 remodeling occurs in the well-controlled goat AF remodeling system (89), consistent with clinical evidence that connexin-40 gene variants predispose to AF (90, 91). The importance of atrial connexin-43 dephosphorylation and lateralization in CHF is unclear, since CHF-induced conduction slowing and AF promotion remain following CHF recovery, despite full resolution of connexin abnormalities (92).

Sodium current (I_{Na}) is a primary determinant of conduction velocity. I_{Na} density is reduced in canine ATR, with corresponding decreases in Nav1.5 subunit mRNA and protein expression (64). Gaborit et al. did not observe atrial I_{Na} changes at the genomic level in AF patients (19). Atrial myocytes from AF patients show either unchanged (93) or only slightly reduced (94) I_{Na}.

Atrial ischemia/infarction produces a substrate for AF via functional reentry circuits stabilized by a line of conduction block in the ischemic zone (39, 40). With acute ischemia, the conduction block is likely related to gap junction uncoupling (40).

Extracardiac factors contributing to functional reentry

Clinical AF is more likely under vagotonic conditions, with AF in some patients clearly vagally dependent (95). Vagal nerve endings release acetylcholine, stimulating cardiac muscarinic cholinergic receptors that activate $I_{K,ACh}$. Vagal innervation is patchy, so the

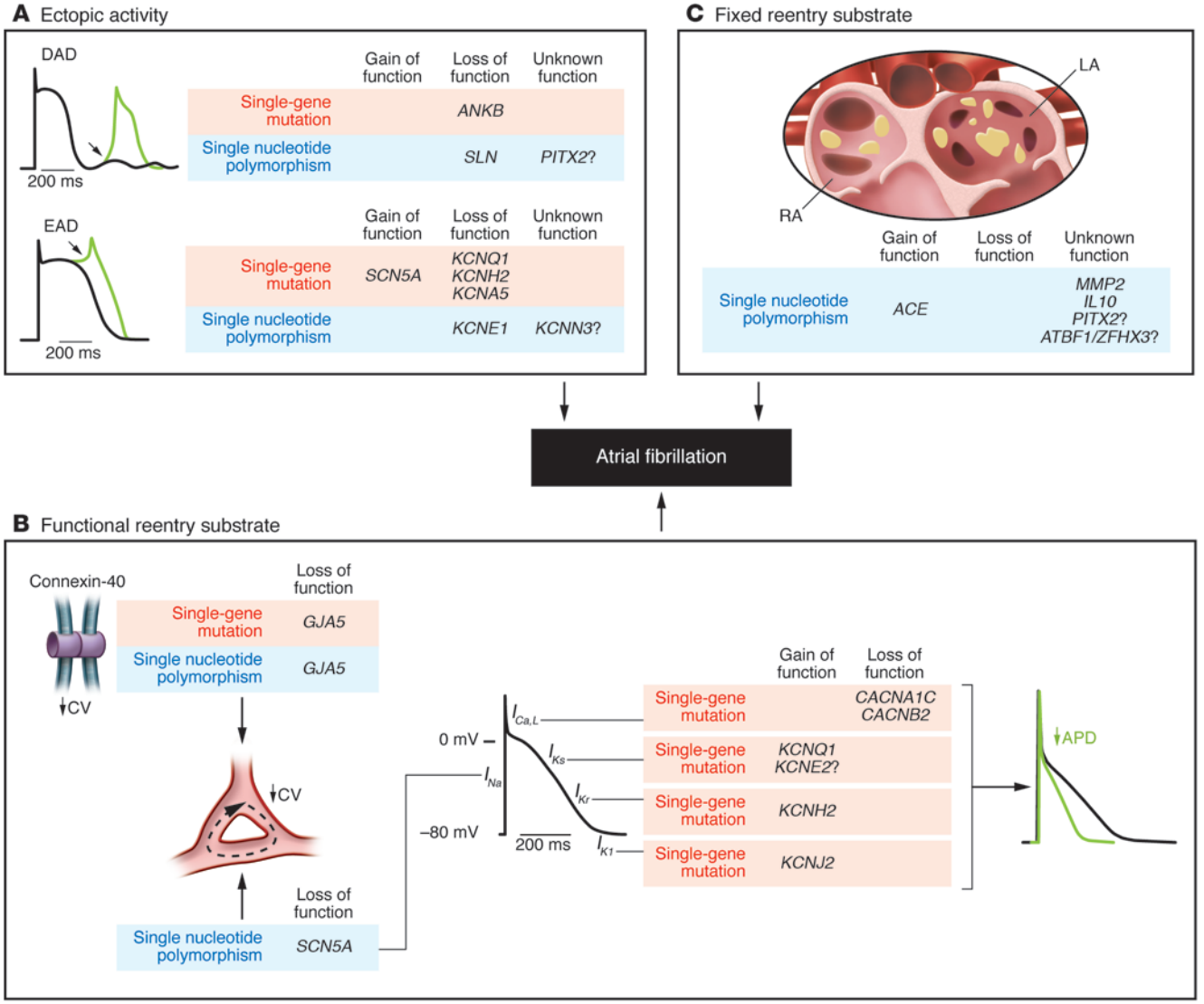

Figure 7
Genetic variants predisposing to AF. Genetic variants (single-gene mutations: red; single nucleotide polymorphisms: blue) are displayed in relation to presumed AF-promoting mechanisms: (**A**) ectopic activity; (**B**) reentry with functional substrates; (**C**) reentry with fixed structural substrates.

effects of vagal activation are spatially heterogeneous, promoting the initiation and stabilization of multiple AF-maintaining reentrant rotors (95). Knockout of the $I_{K,ACh}$ pore-forming subunit Kir3.1 prevents cholinergic AF in mice (96).

Genetic factors contributing to functional reentry

APD shortening. Perhaps the most common AF-promoting genetic paradigm is APD shortening caused by gain-of-function K^+ channel mutations (Figure 7B). The first gene mutation linked to lone AF caused gain of function in the α-subunit (*KCNQ1*) of the slow delayed rectifier I_{Ks} (97). Other genes with AF-inducing gain-of-function K^+ channel mutations include *KCNH2* (98), *KCNJ2* (99), and *KCNE2* (100), corresponding to ion channel subunits of I_{Kr}, I_{K1}, and possibly I_{Ks}, respectively. Loss-of-function mutations in $I_{Ca,L}$ subunits would also be expected to decrease APD and promote AF. In 82 patients with Brugada syndrome/short-QT ECG phenotypes, loss-of-function mutations of the *CACNA1C* and *CACNB2* genes, encoding $I_{Ca,L}$ α- and β-subunits, were associated with AF (101). Patients with short-QT syndromes have reduced APDs and are predisposed to AF (17). Despite the correlation between these pathophysiological mechanisms and functional alterations, many questions remain unanswered; for example, why mutations that appear to be associated with gain of function at the atrial level leave ventricular repolarization unaltered or even delayed (17).

Conduction slowing. Several gene variants promote AF by targeting ion channels that govern conduction velocity. The *GJA5* gene encodes connexin-40, a gap junction ion channel that is particularly important in the atria. Mice lacking connexin-40 demonstrate conduction abnormalities and atrial arrhythmias (102). Missense somatic mutations in *GJA5* were identified in 4 of 15 patients with idiopathic AF (91). *GJA5* promoter sequence variants that decrease gene transcription are associated with increased AF vulnerability (90).

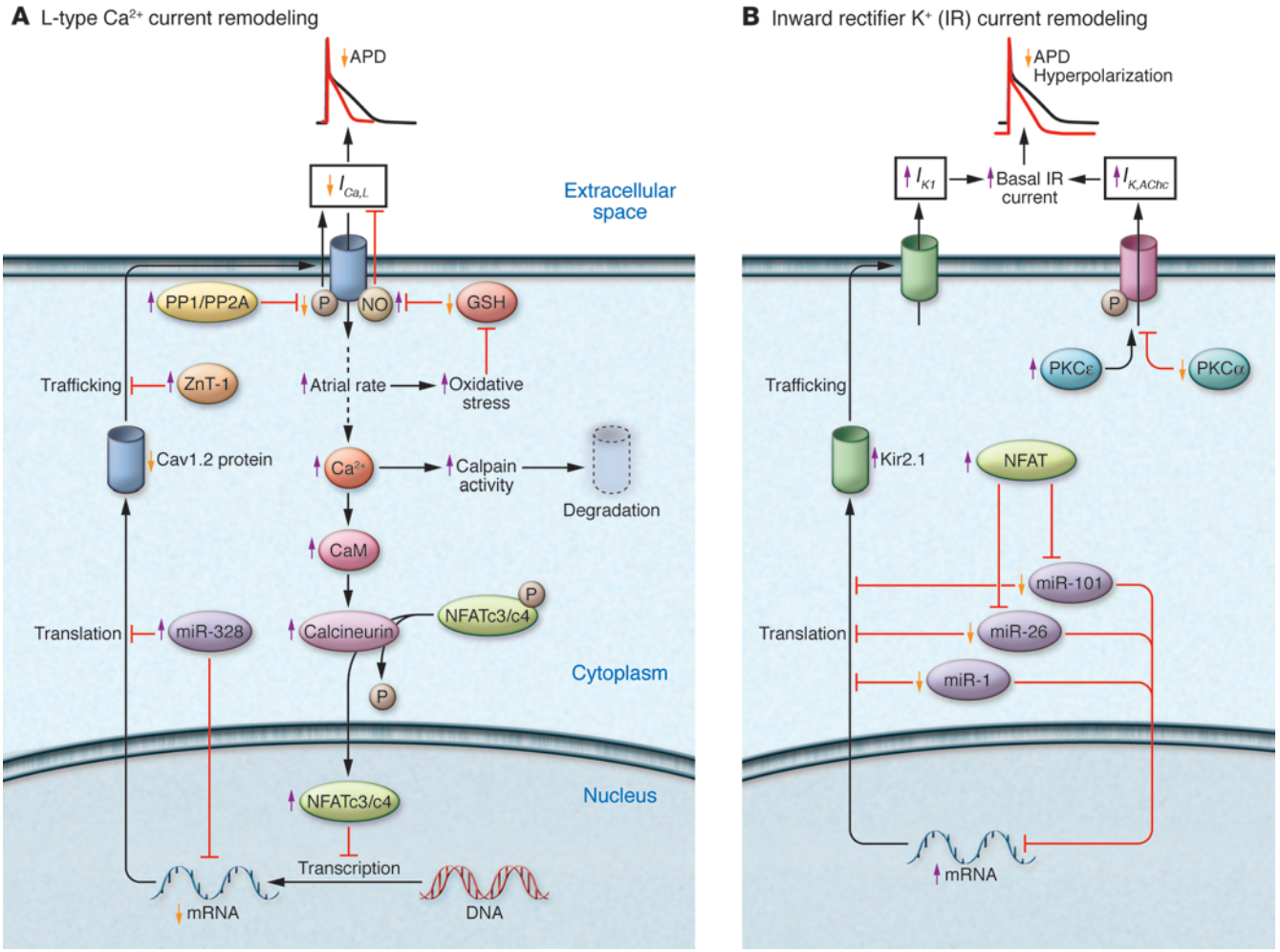

Figure 8
Remodeling of $I_{Ca,L}$ and inward-rectifier K^+ currents by AF/tachycardia. (**A**) The high atrial rate in AF increases intracellular Ca^{2+} load, activating calcineurin via the Ca^{2+}/calmodulin system. Calcineurin stimulates nuclear translocation of nuclear factor of activated T lymphocytes (NFAT), reducing transcription of the principal $I_{Ca,L}$ subunit, Cav1.2. Increased mRNA degradation/impaired protein translation of Cav1.2 and breakdown of Cav1.2 protein by calpains may also contribute. Increased expression of zinc transporter–1 (ZnT-1) impairs membrane trafficking of Cav1.2. Reduced Cav1.2 phosphorylation due to increased protein phosphatase (PP) activity and increased channel nitrosylation may also decrease $I_{Ca,L}$. GSH, glutathione. (**B**) Increased I_{K1} density results from upregulation of the principle Kir2.1 subunit, likely due to reduced levels of inhibitory miRNAs (miR-101, miR-26, miR-1). Increased $I_{K,AChc}$ is caused by altered PKC regulation: increased membrane abundance of stimulatory PKCε and reduced expression of inhibitory PKCα.

Gene variants impairing Na^+ channel function promote AF, presumably via conduction slowing that favors reentry. Loss-of-function Na^+ channel α-subunit (*SCN5A*) mutations were first associated with AF in a family with dilated cardiomyopathy, AF, impaired automaticity, and conduction slowing (103). Subsequently, additional loss-of-function *SCN5A* mutations and SNPs were identified in idiopathic AF subjects (104, 105). Loss-of-function *SCN5A* mutations are the most common genetically defined cause of Brugada syndrome, which classically produces sudden death due to ventricular fibrillation, but which also frequently causes AF (106). More recently, loss-of-function mutations in the cardiac Na^+ channel β-subunits *SCN1B*, *SCN2B*, and *SCN3B* have been associated with AF (107, 108).

AF has been associated with a SNP of the *NOS3* gene encoding eNOS (109). Experimental data suggest that eNOS can regulate cardiac vagal activity and $I_{Ca,L}$, providing plausible links to reentry substrates (110, 111).

Molecular mechanisms leading to a fixed substrate for reentry

Atrial fibrosis plays an important role in many AF-promoting pathologies (10). Once formed, atrial fibrosis reverses slowly if at all (10); prevention therefore becomes very important. Considerable effort is being invested in targeting mechanisms leading to atrial fibrosis to develop effective prophylactic therapy (112).

Heart disease–related atrial fibrosis

Atrial fibrosis occurs in many pathophysiological settings, including senescence, cardiac dysfunction, valve disease, and ischemic heart disease (10, 39, 113, 114). Profibrotic signaling pathways

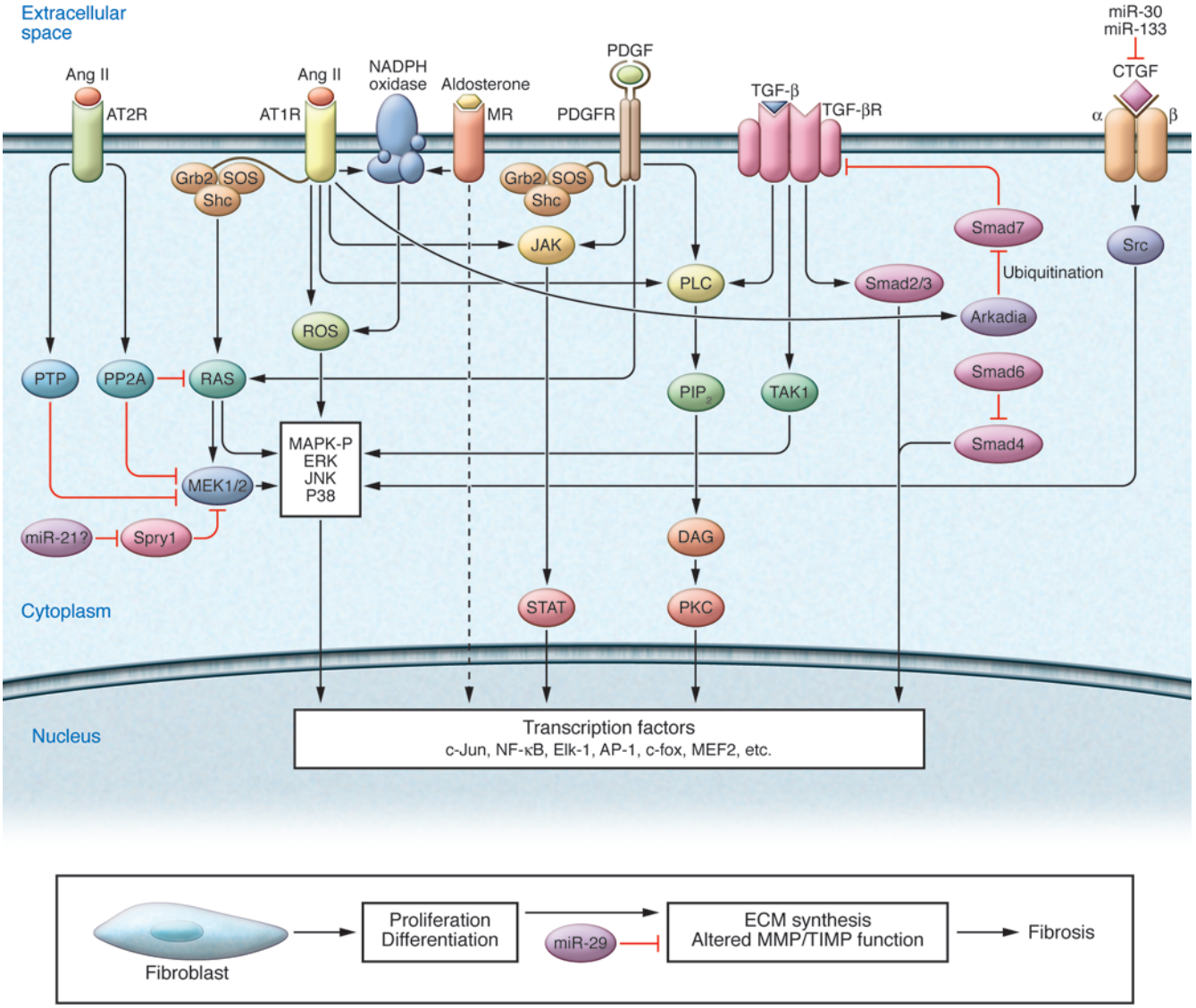

Figure 9
Molecular mechanisms leading to atrial fibrosis. Major profibrotic signaling pathways involved in fibrosis generation are shown. MR, mineralocorticoid receptor.

(Figure 9) demonstrate extensive crosstalk; fibrosis likely results from multiple factors working simultaneously rather than any single individual component. There are extensive interactions between cardiomyocytes and fibroblasts (10). Fibroblasts produce fibrotic ECM proteins and cardioactive mediators that affect cardiomyocyte phenotype, whereas cardiomyocyte-derived products such as ROS, TGF-β, PDGF, and connective tissue growth factor (CTGF) modulate fibroblast properties and ECM synthesis.

Ang II, a well-characterized profibrotic molecule, plays an important role in AF (115). Ang II type 1 receptors (AT1Rs) have profibrotic actions mediated via enhanced expression of TGF-β, Smad2/3, Smad4, Arkadia, and activated ERK MAPK (116). Arkadia reduces Smad7 expression by marking it for ubiquitination, increasing TGF-β signaling by removing Smad7 antagonism at the TGF-β receptor (117). In addition, AT1R signaling through the Shc/Grb2/SOS adapter-protein complex activates the GTPase protein Ras, which enhances profibrotic MAPK phosphorylation (118). AT1R stimulation activates phospholipase C (PLC), further acting through phosphatidylinositol 4,5-bisphosphate (PIP_2), diacylglycerol (DAG), and IP_3. DAG activates PKC, while IP_3 causes intracellular Ca^{2+} release, both contributing to remodeling. The AT1R-stimulated JAK/STAT pathway activates transcription factors such as activating protein–1 (AP-1) and NF-κB, which promote cardiomyocyte remodeling. On the other hand, AT2R activation counteracts MAPK activation by increasing dephosphorylation through PP2A and phosphotyrosine phosphatase (PTP) (118).

$TGF\text{-}\beta_1$, secreted by both fibroblasts and cardiomyocytes, is an important profibrotic mediator (119). Cardiac overexpression of constitutively active $TGF\text{-}\beta_1$ causes selective atrial fibrosis, conduction heterogeneity, and AF susceptibility (120). $TGF\text{-}\beta_1$ functions as a downstream mediator of Ang II in both paracrine and autocrine ways (119). It acts primarily through the SMAD pathway to stimu-

late fibroblast activation and collagen production (10, 117). Rapid atrial firing causes atrial cardiomyocytes to produce substances, recently identified as Ang II and ROS acting via enhanced TGF-β production, that cause fibroblast differentiation into ECM-secretory myofibroblasts (121, 122). This effect likely contributes to progressive structural remodeling during long-standing AF.

PDGF stimulates proliferation and differentiation of fibroblasts (10, 119). PDGF receptors contain two transmembrane domains, which dimerize on stimulation and activate a tyrosine kinase. This tyrosine kinase autophosphorylates other intracellular domains that activate the PDGF receptor and initiate signaling via Ras/MEK1/2 and MAPK, JAK/STAT, and PLC. PDGF overexpression causes cardiac fibrosis and cardiomyopathy (123). PDGF is also secreted by mast cells, which may contribute to profibrotic fibroblast responses associated with low-grade atrial inflammation (124). The genes encoding PDGF and its receptor are more strongly expressed in atrial than ventricular fibroblasts, and PDGF receptor tyrosine kinase inhibition suppresses atrial fibroblast hyperresponsiveness, suggesting that PDGF may contribute to the more intense fibrotic responses of atria versus ventricles (119).

CTGF, a member of the CCN (cyr61/ctgf/nov) protein family, is a downstream mediator of TGF-β_1 and Ang II profibrotic signaling. CTGF activates fibroblasts via Src kinase and MAPKs (125). Genomic analysis points to CTGF as a central player in atrial structural remodeling (126). Recent work implicates CTGF as a key signaling molecule downstream of Rac1 (127).

miRNAs are emerging as potentially important players in atrial fibrosis. miR-29 targets and inhibits collagen genes (128). miR-29 downregulation in atrial tissue precedes and likely contributes to profibrillatory atrial fibrosis (129). Similarly, miR-30 and miR-133, which suppress CTGF production (130), are downregulated in the AF-promoting, atrial profibrotic CHF substrate (131). Atrial miR-21 is upregulated in CHF (131). Elegant work suggests profibrotic effects of miR-21 via suppression of sprouty 1 (*Spry1*), which enhances fibroblast MAPK phosphorylation and survival (132); however, these findings have recently been questioned (133).

Extracardiac factors contributing to atrial fibrosis

Patients with sleep apnea, obesity, and diabetes are at increased risk of AF (15), but exploration of the underlying mechanisms has just begun. Sleep apnea increases atrial pressure, causing atrial stretch that could promote remodeling (134). Diabetes causes atrial fibrosis, along with increased expression of the receptor for advanced glycosylation end-products (AGEs) (RAGE) and CTGF (135). Suppression of AGE production prevented diabetes-related atrial remodeling and CTGF upregulation in a rat model (135). Adrenergic stimulation activates MAPKs and profibrotic remodeling paradigms; however, no clear role has yet been demonstrated in atrial fibrotic remodeling.

Genetic factors contributing to atrial fibrosis

SNPs in genes contributing to atrial structural integrity, and affecting inflammation and neurohumoral pathways, have been associated with AF by candidate gene approaches. Noteworthy are polymorphisms in genes encoding angiotensin-converting enzyme (ACE) (136), MMP2, and interleukin-10 (137). GWASs have identified SNPs on chromosome 4q25, with *PITX2c* suspected to be the target gene, and a SNP on chromosome 16q22 near the AT-binding factor (*ATBF*) gene, also known as zinc finger homeobox 3 (*ZFHX3*) (138). In addition to a possible mechanism involving pulmonary vein ectopy, *PITX2c*'s role in cardiac development (57, 58) raises the possibility that its dysfunction could cause atrial structural remodeling. *ATBF/ZFHX3* is a tumor-suppressor gene (139) and promotes survival of neurons by inducing PDGF receptor β expression and protecting against oxidative stress (140). These properties are consistent with a role in atrial structural remodeling processes.

Potential therapeutic implications

AF is a problem of growing proportions that has been termed "epidemic" (141). Present therapeutic options have limited efficacy and disconcerting adverse-effect risks (2, 3). Consequently, a variety of novel, mechanism-based approaches are in development (2). One approach has been to target atrial remodeling, with the use of "upstream therapy" interventions that interfere with putative signaling pathways. Interventions such as statin drugs, omega-3 fatty acids, and renin-angiotensin system inhibitors prevent atrial remodeling in experimental models (142). While retrospective analyses of clinical data have been promising, the few prospective randomized clinical trials completed to date have been negative or inconclusive (142). Even though the notion of remodeling prevention is very attractive, its feasibility remains to be demonstrated.

Knowledge of AF mechanisms may allow for more direct targeting of specific pathophysiological contributors. The importance of APD as a determinant of reentry led to widespread use of APD-prolonging "class III" agents in AF; however, most of these agents target I_{Kr}, and their use is limited by potentially life-threatening ventricular arrhythmia induction due to EAD mechanisms (2). Several approaches are being studied to treat AF by prolonging atrial APD without affecting the ventricles. One is to target $I_{K,ACh}$, which is prominently involved in AF (70, 95) but absent in the ventricles. A highly selective $I_{K,ACh}$ inhibitor has recently been shown effective in several experimental AF models (143). Another mechanism-based target under study is the Ca^{2+}-dependent K^+ current (I_{KCa}), which appears to be important in AF (59–61). A selective small-conductance I_{KCa} channel inhibitor suppresses AF in a range of animal models (144). Still another approach is to use gene transfer to deliver dominant-negative K^+ channel subunits to selectively prolong atrial APD, which suppresses AF in a porcine model (145).

Ectopic activity due to diastolic RyR2 Ca^{2+} release is also a potential target. Stabilizers of RyR2 show value in AF prevention (146, 147). CaMKII hyperphosphorylation plays a role in AF-related RyR2 dysfunction, and interventions targeting CaMKII hold promise (6).

The emerging role of miRNAs in AF-inducing atrial remodeling presents potentially exciting therapeutic opportunities. miRNAs are stable in blood, and interventions have been developed to enhance or suppress the expression of miRNAs involved in disease progression (148). The apparent participation of miRNAs in a wide range of AF-inducing mechanisms, including abnormal SR Ca^{2+} release, APD reduction, connexin modulation, and tissue fibrosis, points to potential target mechanisms (77).

Recent insights into the molecular pathophysiology of AF open new opportunities in risk assessment and monitoring of therapeutic responses. Novel biomarkers under investigation include noninvasive indices of atrial fibrosis (149) and plasma biomarkers reflecting underlying biochemical mechanisms or responses (150).

A great deal of information about the molecular pathophysiology of AF has been obtained over the past 10 years. This information has provided insights, raised many new questions, and provided novel therapeutic opportunities. It is hoped that concrete improvements in AF management will result, but only time and a great deal of additional research will tell.

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