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Altered Atrial Metabolism: An Underappreciated Contributor to the Initiation and Progression of Atrial Fibrillation

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Atrial fibrillation (AF) is the most common cardiac arrhythmia and is associated with increased morbidity and mortality. Currently, about 1% to 2% of the general population suffers from AF.[1] The incidence and prevalence of AF increase in an age-dependent manner, resulting in a projected AF-prevalence in the United States in 2050 of ≈16 millions.[1] It is assumed that between a quarter and two-thirds of the AF population has transient or paroxysmal AF (pAF, defined as AF self-terminating within 48-hours) and up to 35% of patients have silent (non-symptomatic) AF.[1] In addition, each year up to 15% of initially diagnosed pAF patients will progress to persistent (AF persisting >7 days or requiring termination by cardioversion) or permanent (attempts to achieve sinus rhythm are abandoned) forms.

AF can have serious clinical complications, with heart failure, stroke, and sudden cardiac death being the most prominent. The mechanisms of AF initiation and/or progression to both more persistent forms, as well as mechanisms promoting life-threatening complications are poorly understood and are unpredictable in an individual patient.[2,3] Accordingly, currently available drug treatments for AF are poorly effective and cause substantial proarrhythmia.[4] Modern ablation procedures are effective in specific AF populations and generally show superior anti-AF efficacy compared with antiarrhythmic drugs,[5] but due to the very large size of the AF population, it is unlikely that ablation can be applied to the majority of AF patients. Therefore, treatment with antiarrhythmic drugs will likely remain the mainstay therapy for AF and there is a clear unmet need for novel antiarrhythmic drugs.

The limited efficacy of present antiarrhythmic drugs likely results from the fact that they were not developed based on a precise understanding of the fundamental arrhythmia mechanisms underlying AF initiation and progression.[4] The precise understanding of these mechanisms is expected to help to design novel antiarrhythmic drugs with increased efficacy and improved safety profiles. Despite the clear need for further investigative research of AF, currently employed large-animal models have serious limitations with respect to the extrapolation of abnormalities to human AF.[6] To the best of our knowledge, no good experimental model for the spontaneous AF initiation exists. In most models, AF is artificially initiated through pacing. Although the subsequent AF duration may provide information about the mechanisms maintaining AF, AF initiation cannot be studied in these models. Similarly, some models maintain a rapid atrial activation over prolonged periods using atrial pacing, thereby providing information about the AF consequences, but do not allow assessment of initiation mechanisms. A better understanding of the specific atrial alterations that predispose to AF initiation may lead to more tailored therapeutic approaches. Transgenic mouse models have been instrumental to study the consequences of a specific atrial abnormality,[6,7] but only a few mouse models develop spontaneous AF episodes. One notable exception is the CREM-IbΔC-X transgenic mouse model that spontaneously develops AF episodes and shows a progression in AF duration.[8]

In this issue, Ozcan et al[9] show that cardiomyocyte-specific ablation of liver kinase B1 (LKB1), an activator of AMP-activated serine/threonine protein-kinase (AMPK) involved in cell metabolism,[10] results in the development of spontaneous AF in an age-dependent manner. In addition, LKB1 knockout (LKB1-KO) mice show an age-dependent progression of AF from a paroxysmal to a persistent form, thus mimicking the disease progression observed in many AF patients. At the time of AF detection, LKB1-KO mice showed age-dependent atrial structural remodeling characterized by bi-atrial enlargement with inflammation, heterogeneous fibrosis with reduced connexin-40 and connexin-43 expression, along with disrupted cell ultrastructure, apoptosis, and necrosis. These findings suggest that LKB1 ablation and the related metabolic abnormalities may promote reentrant arrhythmogenesis by promoting the

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development of a susceptible substrate involving structural remodeling and connexin-downregulation-mediated conduction slowing (Figure). Like in patients, persistent AF was accompanied by left ventricular dysfunction, heart failure, ventricular fibrillation, and reduced survival. All atrial alterations in LKB1-KO mice were associated with increased oxidative stress indicated by elevated H_2O_2 levels and a decreased AMPK phosphorylation/activity. These findings point to a potential causal role for metabolic abnormalities and oxidative stress related to the LKB1/AMPK pathway in atrial structural remodeling and conduction slowing, contributing to the development of a substrate for spontaneous AF and the progression to persistent AF in LKB1-KO mice.

The present work has multiple implications. It suggests that altered atrial metabolism and increased oxidative stress may be essential components in both AF initiation and progression. Proper atrial contraction depends on precisely regulated excitation-contraction coupling and relaxation. These processes consume a huge amount of cellular energy, which is produced by oxidative phosphorylation in mitochondria. In addition, the majority of ion channels and transporters involved in excitation-contraction coupling, as well as protein kinases and phosphatases, which control reversible protein phosphorylation, are sensitive to redox-state.[11] Therefore, it is not surprising that oxidative stress and altered atrial metabolism are involved in the development of proarrhythmic atrial remodeling. Consistent with this idea, the most prominent differences between pAF and sinus rhythm patients relate to the expression of proteins involved in metabolic processes, suggesting atrial metabolic disturbances as major contributors to the spontaneous AF initiation.[12]

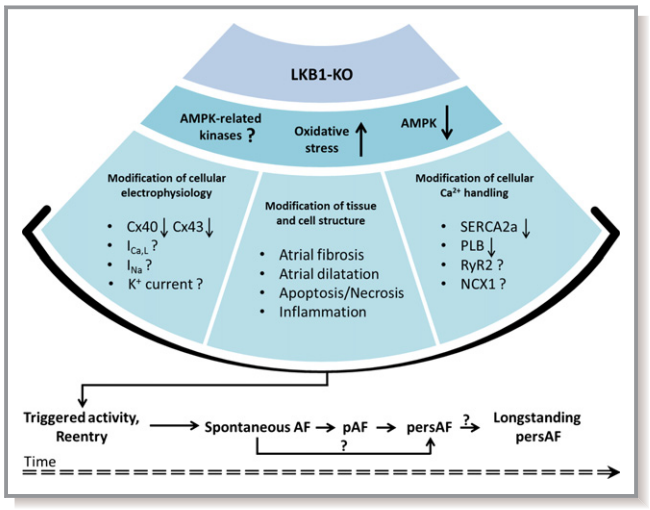

Figure. Schematic of putative consequences of LKB1 ablation, which could play a role in the initiation of spontaneous AF and in the progression to persistent AF. Loss of LKB1 inactivates AMPK and perhaps AMPK-related kinases and causes oxidative stress. These changes might cause alterations in atrial electrophysiology and structure, and in cellular Ca^{2+} handling, creating a vulnerable substrate for triggered activity and reentry, promoting spontaneous AF and the gradual, age-dependent transition from atrial ectopy to paroxysmal AF (pAF) and persistent AF (PersAF). AF indicates atrial fibrillation; AMPK, AMP-activated serine/threonine protein-kinase; Cx, connexin; $I_{Ca,L}$, L-type Ca^{2+}-current; I_{Na}, Na^+-current; LKB1-KO, liver kinase B1 knockout; NCX1, Na^+-Ca^{2+}-exchanger type-1; PLB, phospholamban; RyR2, ryanodine receptor channel type-2; SERCA2a, sarcoplasmic reticulum Ca^{2+}-ATPase type-2a.

Oxidative stress is an established contributor to triggered activity as well as potentially promoting the transition from pAF and persistent AF.[13] Triggered activity results from an increased incidence of spontaneous Ca^{2+}-release events (Ca^{2+}-leak) from the sarcoplasmic reticulum (SR) via ryanodine receptor channels type-2 (RyR2). Potentially proarrhythmic SR Ca^{2+}-release events result from an increased RyR2 open probability due to abnormal oxidation/phosphorylation, or from SR Ca^{2+} overload due to increased SR Ca^{2+} uptake via the SR Ca^{2+}-ATPase type-2a (SERCA2a).[14,15] Oxidative stress increases RyR2-function either through direct RyR2 oxidation or via an oxidation-mediated activation of Ca^{2+}/calmodulin-dependent protein kinase-II (CaMKII), which causes RyR2-hyperphosphorylation.[16,17] Although SERCA2a-expression is strongly downregulated in LKB1-KO mice,[18] which should reduce SR Ca^{2+} load, hyperoxidation of CaMKII and RyR2 might cause SR Ca^{2+} leak leading to atrial ectopy, predisposing to spontaneous AF. However, it needs demonstration that triggered activity and atrial ectopy contribute to atrial arrhythmogenesis in LKB1-KO mice.

Ablation of LKB1 caused a reduction in AMPK Thr172-phosphorylation and thus in its enzymatic activity. AMPK modulates downstream signaling to maintain cellular energy homeostasis by increasing energy availability and decreasing energy expenditure,[19] promoting oxidative metabolism. LKB1 is the principal kinase phosphorylating AMPK-Thr172 and loss of LKB1 limits AMPK activation.[18] Preliminary results show that Thr172-phosphorylation of AMPK is higher in pAF patients, but lower in patients with persistent AF,[20] pointing to the possibility that reduced AMPK activity may support AF persistence. Thus, reduced AMPK Thr172-phosphorylation may be a key mechanism promoting the observed atrial changes and the subsequent transition from paroxysmal to persistent AF in LKB1-KO mice and could represent potential biomarker of AF-persistence. Here, all LKB1-KO mice which developed pAF progressed to persistent AF, whereas in the clinical setting only a minority of pAF-patients progress to persistent/permanent AF, suggesting that LKB1-KO mice are a model for one specific population of AF patients. Further extensive work is needed to prove and validate the role of AMPK as a contributor to AF initiation and a biomarker for AF persistence.

Classical electrical remodeling (abbreviated effective refractory period and hyperpolarized resting membrane potential due to ionic current changes) was not studied in the present mouse model. Although electrical remodeling might contribute to the

proarrhythmic phenotype in LKB1-KO mice, the contribution of electrical remodeling in patients is currently uncertain because most AF episodes terminate spontaneously despite the presumed development of electrical remodeling during the first days of AF. Also, electrical remodeling could be considered an adaptive mechanism to counteract cellular Ca^{2+} overload and related remodeling during AF.[3] In the current study, structural remodeling appears to be sufficient for both the initiation and progression of AF, making classical electrical remodeling an unlikely causal factor.

The present model of spontaneous AF recapitulates key clinical predictors of AF induction (atrial flutter, atrial dilatation, inflammation, fibrosis, etc), offering the unique opportunity to investigate the precise molecular changes associated with each of these risk factors during the initiation and progression of AF. One previous study using the same mouse model showed that these structural changes are present in atria of LKB1-KO mice at the time of first AF detection.[18] Thus it is very likely that the atrial changes due to LKB1 ablation predispose to AF initiation by creating a vulnerable arrhythmogenic substrate (Figure), although the bidirectional interactions between atrial remodeling due to metabolic abnormalities and AF requires thorough investigation in subsequent work.

In conclusion, the importance of the evolution of AF from more tractable to resistant forms is a widely recognized clinical challenge.[13] The most important obstacle to effective long-term AF management is the progression of the substrate, as manifested by substantial late AF recurrence rates after initially effective AF ablations. Ultimately, the only way to prevent long-term AF recurrence will be the development of novel effective methods to forestall substrate progression. The results of Ozcan et al[9] are very significant in this regard, because they provide new insights into the role of atrial metabolism for AF substrate evolution, which may ultimately lead to improved therapeutic approaches to prevent AF progression.

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Disclosures

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