Published in final edited form as:
*Circulation*. 2013 October 15; 128(16): 1724–1726. doi:10.1161/CIRCULATIONAHA.113.005837.

# Oxidative Stress and Atrial Fibrillation: Finding a Missing Piece to the Puzzle

**Kai-Chien Yang, MD, PhD** and **Samuel C. Dudley Jr., MD, PhD**
Lifespan Cardiovascular Institute, the Warren Alpert School of Medicine, Brown University, and the Providence Veterans Administration Medical Center, Providence, RI

**Keywords**

Editorial; oxidative stress; calcium/calmodulin-dependent protein kinase II; NAD(P)H oxidase; arrhythmia (heart rhythm disorders)

Atrial fibrillation (AF), one of the most prevalent cardiac arrhythmias, afflicts more than 3 million people in the United States.[1] As the risk of developing AF increases with age, the prevalence of AF is projected to be 7.6 million in 2050,[1] when one in five Americans will be over the age of 65.[2] AF not only causes debilitating symptoms and functional impairments owing to worsened hemodynamics and embolic stroke, it also increases the risk of mortality up to 1.5–1.9 fold.[3] Despite the significant morbidity and mortality burden incurred with AF, there are limited therapeutic options that may improve the outcomes of AF patients, and these options are associated with significant AF recurrence rates.[4]

One possible explanation for the limitations of current therapies is that they do not address effectively or completely the underlying causes of AF. Evidence has been mounting that AF is associated with systemic and cardiac oxidation.[4,5] Risk factors for AF are similar to those of atherosclerosis, a disease known to be perpetuated by oxidative stress. These risk factors, such as hypertension, aging, diabetes and coronary artery bypass surgery, have each been associated with increases systemic markers of oxidation.[4] In addition, there is also evidence of increased cardiac oxidation of myofibrillar protein[5] and membrane lipids[6] with AF or with risk factors linked to AF. While it is not clear whether the cardiac oxidation leads to systemic markers of oxidation or whether systemic oxidation leads to cardiac oxidation, the association of oxidative stress and AF is robust and suggests that AF is possibly a manifestation of a systemic disease.

Despite the link between oxidative stress and AF, systemic antioxidant therapy for arrhythmias has not met with much success in clinical trials.[7,8] This suggests that the oxidative stress is either not in the pathogenic cascade of arrhythmogenesis or our current antioxidant therapies are not targeting the pathogenic source of oxidative stress in arrhythmia. Consistent with the later hypothesis, a recent paper by Sovari et al.[9] showed that

Address for Correspondence: Samuel C. Dudley, Jr., MD, PhD, Director, Lifespan Cardiovascular Institute, Ruth and Paul Levinger Chair in Medicine, The Warren Alpert Medical School of Brown University, 593 Eddy Street, APC 730, Providence, RI 02903, Phone: 401-444-5328, Fax: 401-444-4652, samuel_dudley@brown.edu.

**Conflict of Interest Disclosures:** SCD is an inventor of 7,550,299 Method for predicting onset/risk of atrial fibrillation (AF), 8,003,324 Modulation of sodium channels by nicotinamide adenine dinucleotide, 11/882,627 Method for predicting onset/risk of atrial fibrillation (AF), 12/929,786 Method for Modulating or Controlling Sodium Channel Current by Reactive Oxygen Species, 13/032,629 Activation of the Renin-Angiotensin System (RAS) and Sudden Cardiac Death, 13/551,790 Method for Ameliorating or Preventing Arrhythmic Risk Associated with Renin-Angiotensin System Activation, and 13/507,319 Method for Modulating or Controlling Connexin 43 (Cx43) Level of a Cell. There is no disclosure for KCY.

angiotensin II (AngII)-induced ventricular arrhythmias could only be prevented by a mitochondria-targeted antioxidant rather than a general antioxidant or inhibitors of other producers of oxidative stress.

In this issue of *Circulation*, Purohit and colleagues[10] conducted an elegant study using human tissues and mouse models to demonstrate the crucial role of oxidized $Ca^{2+}$ and calmodulin-dependent protein kinase II (ox-CaMKII) in mediating AngII/pacing-induced AF. Purohit et al. present strong, new evidence that oxidative stress can lead to AF. The proposed mechanistic link between renin-angiotensin system (RAS) activation-induced oxidative stress, intracellular $Ca^{2+}$ handling and the inducibility of AF also helps to explain the antiarrhythmic effects of angiotensin converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs).

## AngII-mediated CaMKII oxidation promotes sarcoplasmic reticulum (SR) $Ca^{2+}$ leak, potentiating pacing-induced AF

Recent works by the Anderson group have identified that the oxidation of a pair of methionines (281/282) in the CaMKII regulatory domain prevents the inactivation of CaMKII[11] and that this constitutively active ox-CaMKII is responsible for AngII-induced sinoatrial node dysfunction[12] and aldosterone-mediated cardiotoxicity.[13] In this work by Purohit et al.,[10] they demonstrated that ox-CaMKII expression was increased in the atrial tissue from patients with AF, compared with those in sinus rhythm, without affecting total CaMKII levels. Interestingly, the increase in atrial ox-CaMKII is not observed among AF patients treated with an ACEI or ARB. Using a mouse model of burst pacing-induced AF, they showed that three weeks of AngII infusion, a treatment known to increase atrial ox-CaMKII levels,[12] significantly increased the susceptibility to AF induction. Using the CaMKIIδ oxidation resistant knock-in mice (MM-VV), they showed that this AngII-potentiated AF induction by burst pacing requires the presence of ox-CaMKIIδ, whereas the reactive oxygen species (ROS) production, hypertension and cardiac hypertrophy in response to AngII treatment were unaffected in MM-VV mice. In addition, using mouse models lacking functional NADPH oxidase ($p47^{-/-}$) or cardiac-restricted expression of methionine sulfoxide reductase (MsrA), they went on to show that NADPH oxidase-dependent ROS production and elevated ox-CaMKII are essential for the proarrhythmic effects of AngII in pacing-induced AF.

To determine if increased SR $Ca^{2+}$ leak contributes to AngII-potentiated AF susceptibility, they measured the diastolic $Ca^{2+}$ sparks in isolated atrial myocytes, where the spontaneous $Ca^{2+}$ sparks and delayed afterdepolarizations (DADs) significantly increased in atrial myocytes from AngII-treated, compared to saline-treated, wild-type mice. AngII-treated MM-VV mice did not show an increase in $Ca^{2+}$ sparks or DADs, suggesting the requirement of ox-CaMKII in AngII-induced SR $Ca^{2+}$ leak and DADs. Finally, they demonstrated that the proarrhythmic effects of AngII treatment on pacing-induced AF can be abolished in the mice expressing modified RyR2 (S2814A), which is resistant to CaMKII-mediated phosphorylation, as well as in the mouse model with cardiac-specific expression of a CaMKII inhibitory peptide (AC3-I), suggesting the critical role of CaMKII-mediated RyR2 phosphorylation at S2814 for AF inducibility in response to AngII treatment and burst pacing.

## Oxidized-CaMKII-induced SR $Ca^{2+}$ leak: Another missing piece of the complex puzzle

The results by Purohit et al.[10] are consistent with previous reports showing that diastolic SR $Ca^{2+}$ leak is increased in atrial myocytes from human and animals with AF.[14] In a recent work by Neef et al.,[15] increased $Ca^{2+}$ leak from SR and elevated diastolic $Ca^{2+}$ concentration were observed in the atrial myocytes from patients with AF, which is associated with increased CaMKII expression and increased RyR2 phosphorylation at S2814. CaMKII inhibition normalizes SR $Ca^{2+}$ leak and cytosolic $Ca^{2+}$ levels without affecting L-type $Ca^{2+}$ currents. Increased phosphorylation of RyR2 by CaMKII was also reported to be responsible for increased SR $Ca^{2+}$ leak, DADs and pacing-induced AF in mice.[16] The findings by Purohit et al.[10] add to the existing evidence, suggesting the importance of CaMKII-induced RyR2 phosphorylation and increased SR $Ca^{2+}$ leak in the pathogenesis of AF. Importantly, this elegant work uncovers the novel role of CaMKII as the redox sensor downstream of AngII to transduce elevated ROS production into SR $Ca^{2+}$ dysregulation and increased AF susceptibility.

Although increased SR $Ca^{2+}$ leak from RyR2, as shown by Purohit et al.[10] and others,[14,15] appears to be an attractive mechanism contributing to AF by triggering DADs, this mechanism by itself will only increase diastolic $Ca^{2+}$ concentration transiently, owing to the inevitable SR $Ca^{2+}$ load depletion.[17] It is possible that other $Ca^{2+}$ handling proteins, including sarcoplasmic reticulum $Ca^{2+}$ ATPase (SERCA), phospholamban (PLB) or L-type $Ca^{2+}$ channels, are concomitantly modulated in this AngII/pacing-induced AF model to replenish SR $Ca^{2+}$ load, thereby sustaining the SR $Ca^{2+}$ leak and elevated diastolic $Ca^{2+}$ levels. Indeed, PLB, the endogenous inhibitor of SERCA in its unphosphorylated state, is known to be hyperphosphorylated at Thr-17 by CaMKII in atrial myocardium of AF patients, leading to increased SERCA activity and reuptake of $Ca^{2+}$ into the SR.[18] It would be of great interest to see if ox-CaMKII also plays a role in regulating these calcium regulatory proteins in AF patients as well as in the AngII/pacing-induced AF mouse model.

## Oxidative stress and AF: A complex interplay

In the data presented by Purohit et al., inhibition of CaMKII oxidation by ACEI or ARB did not prevent all AF. This is consistent with the idea that there are more pathways and mechanisms involved than the one outlined by Purohit. The partial success of ablation therapy focused on creating lines of electrical conduction block in humans also suggests that CaMKII-enhanced triggered activity is not the whole mechanistic answer for AF, since this therapy likely affects reentry as wells as arrhythmogenic foci in places such as the pulmonary veins. Other effects of AngII and oxidative stress include alterations in $Na^{+}$ current and connexins can also contribute to forming the AF substrate. Changes in these proteins are mediated by PKC[19] and c-Src,[20] respectively. Mitochondrial dysfunction, associated with ROS release, enhances $K_{ATP}$ channel activity further inhibiting conduction and creating the substrate for reentrant arrhythmias.[21] Mitochondria-targeting antioxidants and c-Src inhibitors, therefore, may prove to be clinically useful antiarrhythmic agents in the future.

The data by Purohit et al. point to the NADPH oxidase as a source of oxidative stress causing AF. Nevertheless, results from Reilly et al.[22] show that this enzyme is downregulated over time in AF, and the Statin Therapy for the Prevention of AF (SToP AF) trial[8] failed to show an effect of atorvastatin, a known inhibitor of the NADPH oxidase, to lower systemic oxidation or to prevent AF after cardioversion in patients with mostly persistent AF. One way to reconcile the observations of Purohit with the data above is that

the NADPH oxidase may participate early in the initiation of AF and some other process sustains the arrhythmia over time.

While the Purohit idea provides a strong mechanistic link between ROS and AF, it does not explain the concept that AF begets AF, an idea that suggests a positive feedback loop in the maintenance of AF. In addition to structural changes with AF over time that also can be partially remediated by RAS inhibition, an interplay between PKC and mitochondrial ROS where PKC activation induces mitochondrial ROS production[19] and mitochondrial ROS activates PKC[23] is another candidate to explain the clinically demonstrated idea that AF perpetuates itself.

The work of Purohit adds an important piece to our understanding of AF, and each new piece suggests better therapies than we currently use. Important future directions are likely to include understanding what percentage of AF is caused by oxidative stress, what are the sources of ROS in AF, which downstream effectors such as CaMKII, c-Src, and PKC are activated by the different sources and types of ROS, what are the effects of ROS on these and other proteins, and exactly how these ROS-induced changes lead to arrhythmias. Puroit et al. have done the medical community a great service by showing oxidative stress can lead to AF and giving us clear mechanisms to target in the future. This is an important piece of the puzzle.

## Acknowledgments

**Funding Sources:** This work was funded by National Institutes of Health (NIH) Grants RO1 HL1024025, RO1 HL106592 and a VA MERIT grant (SCD), and American Heart Association Midwest Affiliation Postdoctoral Fellowship AHA13POST14380029 (KCY).

## References

1. Naccarelli GV, Varker H, Lin J, Schulman KL. Increasing prevalence of atrial fibrillation and flutter in the United States. Am J Cardiol. 2009; 104:1534–1539. [PubMed: 19932788]
2. Wiener JM, Tilly J. Population ageing in the United States of America: implications for public programmes. Int J Epidemiol. 2002; 31:776–781. [PubMed: 12177018]
3. Benjamin EJ, Wolf PA, D'Agostino RB, Silbershatz H, Kannel WB, Levy D. Impact of atrial fibrillation on the risk of death: the Framingham Heart Study. Circulation. 1998; 98:946–952. [PubMed: 9737513]
4. Sovari AA, Dudley SC Jr. Reactive oxygen species-targeted therapeutic interventions for atrial fibrillation. Front Physiol. 2012; 3:311. [PubMed: 22934062]
5. Mihm MJ, Yu F, Carnes CA, Reiser PJ, McCarthy PM, Van Wagoner DR, Bauer JA. Impaired myofibrillar energetics and oxidative injury during human atrial fibrillation. Circulation. 2001; 104:174–180. [PubMed: 11447082]
6. De Vecchi E, Pala MG, Di Credico G, Agape V, Paolini G, Bonini PA, Grossi A, Paroni R. Relation between left ventricular function and oxidative stress in patients undergoing bypass surgery. Heart. 1998; 79:242–247. [PubMed: 9602656]
7. Castillo R, Rodrigo R, Perez F, Cereceda M, Asenjo R, Zamorano J, Navarrete R, Villalabeitia E, Sanz J, Baeza C, Aguayo R. Antioxidant therapy reduces oxidative and inflammatory tissue damage in patients subjected to cardiac surgery with extracorporeal circulation. Basic Clin Pharmacol Toxicol. 2011; 108:256–262. [PubMed: 21138533]
8. Negi S, Shukrullah I, Veledar E, Bloom HL, Jones DP, Dudley SC. Statin therapy for the prevention of atrial fibrillation trial (SToP AF trial). J Cardiovasc Electrophysiol. 2011; 22:414–419. [PubMed: 20946227]
9. Sovari AA, Rutledge CA, Jeong EM, Dolmatova E, Arasu D, Liu H, Vahdani N, Gu L, Zandieh S, Xiao L, Bonini MG, Duffy HS, Dudley SC Jr. Mitochondria oxidative stress, connexin43

remodeling, and sudden arrhythmic death. Circ Arrhythm Electrophysiol. 2013; 6:623–631. [PubMed: 23559673]

10. Purohit A, Rokita AG, Guan X, Chen B, Koval OM, Voigt N, Neef S, Sowa T, Gao Z, Luczak ED, Stefansdottir H, Behunin AC, Li N, El-Accaoui RN, Yang B, Swaminathan PD, Weiss RM, Wehrens XHT, Song L, Dobrev D, Maier LS, Anderson ME. Oxidized CaMKII triggers atrial fibrillation. Circulation. 2013; 128:XX–XXX.
11. Erickson JR, Joiner ML, Guan X, Kutschke W, Yang J, Oddis CV, Bartlett RK, Lowe JS, O'Donnell SE, Aykin-Burns N, Zimmerman MC, Zimmerman K, Ham AJ, Weiss RM, Spitz DR, Shea MA, Colbran RJ, Mohler PJ, Anderson ME. A dynamic pathway for calcium-independent activation of CaMKII by methionine oxidation. Cell. 2008; 133:462–474. [PubMed: 18455987]
12. Swaminathan PD, Purohit A, Soni S, Voigt N, Singh MV, Glukhov AV, Gao Z, He BJ, Luczak ED, Joiner ML, Kutschke W, Yang J, Donahue JK, Weiss RM, Grumbach IM, Ogawa M, Chen PS, Efimov I, Dobrev D, Mohler PJ, Hund TJ, Anderson ME. Oxidized CaMKII causes cardiac sinus node dysfunction in mice. J Clin Invest. 2011; 121:3277–3288. [PubMed: 21785215]
13. He BJ, Joiner ML, Singh MV, Luczak ED, Swaminathan PD, Koval OM, Kutschke W, Allamargot C, Yang J, Guan X, Zimmerman K, Grumbach IM, Weiss RM, Spitz DR, Sigmund CD, Blankesteijn WM, Heymans S, Mohler PJ, Anderson ME. Oxidation of CaMKII determines the cardiotoxic effects of aldosterone. Nat Med. 2011; 17:1610–1618. [PubMed: 22081025]
14. Hove-Madsen L, Llach A, Bayes-Genis A, Roura S, Rodriguez Font E, Aris A, Cinca J. Atrial fibrillation is associated with increased spontaneous calcium release from the sarcoplasmic reticulum in human atrial myocytes. Circulation. 2004; 110:1358–1363. [PubMed: 15313939]
15. Neef S, Dybkova N, Sossalla S, Ort KR, Fluschnik N, Neumann K, Seipelt R, Schondube FA, Hasenfuss G, Maier LS. CaMKII-dependent diastolic SR $Ca^{2+}$ leak and elevated diastolic $Ca^{2+}$ levels in right atrial myocardium of patients with atrial fibrillation. Circ Res. 2010; 106:1134–1144. [PubMed: 20056922]
16. Chelu MG, Sarma S, Sood S, Wang S, van Oort RJ, Skapura DG, Li N, Santonastasi M, Muller FU, Schmitz W, Schotten U, Anderson ME, Valderrabano M, Dobrev D, Wehrens XH. Calmodulin kinase II-mediated sarcoplasmic reticulum $Ca^{2+}$ leak promotes atrial fibrillation in mice. J Clin Invest. 2009; 119:1940–1951. [PubMed: 19603549]
17. Venetucci LA, Trafford AW, Eisner DA. Increasing ryanodine receptor open probability alone does not produce arrhythmogenic calcium waves: threshold sarcoplasmic reticulum calcium content is required. Circ Res. 2007; 100:105–111. [PubMed: 17110597]
18. El-Armouche A, Boknik P, Eschenhagen T, Carrier L, Knaut M, Ravens U, Dobrev D. Molecular determinants of altered $Ca^{2+}$ handling in human chronic atrial fibrillation. Circulation. 2006; 114:670–680. [PubMed: 16894034]
19. Liu M, Sanyal S, Gao G, Gurung IS, Zhu X, Gaconnet G, Kerchner LJ, Shang LL, Huang CL, Grace A, London B, Dudley SC Jr. Cardiac $Na^{+}$ current regulation by pyridine nucleotides. Circ Res. 2009; 105:737–745. [PubMed: 19745168]
20. Sovari AA, Iravanian S, Dolmatova E, Jiao Z, Liu H, Zandieh S, Kumar V, Wang K, Bernstein KE, Bonini MG, Duffy HS, Dudley SC. Inhibition of c-Src tyrosine kinase prevents angiotensin II-mediated connexin-43 remodeling and sudden cardiac death. J Am Coll Cardiol. 2011; 58:2332–2339. [PubMed: 22093512]
21. Akar FG, Aon MA, Tomaselli GF, O'Rourke B. The mitochondrial origin of postischemic arrhythmias. J Clin Invest. 2005; 115:3527–3535. [PubMed: 16284648]
22. Reilly SN, Jayaram R, Nahar K, Antoniades C, Verheule S, Channon KM, Alp NJ, Schotten U, Casadei B. Atrial sources of reactive oxygen species vary with the duration and substrate of atrial fibrillation: implications for the antiarrhythmic effect of statins. Circulation. 2011; 124:1107–1117. [PubMed: 21844076]
23. Giorgi C, Agnoletto C, Baldini C, Bononi A, Bonora M, Marchi S, Missiroli S, Patergnani S, Poletti F, Rimessi A, Zavan B, Pinton P. Redox control of protein kinase C: cell- and disease-specific aspects. Antioxid Redox Signal. 2010; 13:1051–1085. [PubMed: 20136499]