Aging and Protein Kinase Activation

Is It the Missing Link Between Age and Atrial Fibrillation?

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Atrial fibrillation (AF) is the most common sustained clinical arrhythmia and is associated with substantial morbidity and mortality.[1] Age is a key determinant of AF risk: <2% for individuals <60 years of age, increasing exponentially to >14% by 85 years of age.[1] Whereas older age makes AF more likely, younger age seems protective: congenital heart disease patients with enormous, extensively remodeled atria develop atrial tachyarrhythmias, but AF remains the least common form until 50 years of age.[2]

Article, see p 821

Studies of Mechanisms Underlying Age-Dependent AF Susceptibility

The Table summarizes the principal studies in the literature that address mechanisms underlying aging-related AF. Atrial muscle bundles from humans <14 years of age show smooth conduction, whereas older individuals (40–60 years old) have impaired transverse-impulse propagation because of increased interstitial collagen, leading to zigzag conduction.[3] Optical mapping in beagle hearts confirmed transverse conduction changes in 6- to 10-year-old dogs (equivalent to 40–56-year-old humans), with associated decreases in transverse connexin 43 expression.[4] Atrial electrogram fragmentation and refractoriness prolongation occur in older patients, indicating disturbances in both conduction and repolarization.[5] Electroanatomic studies show slowed conduction and reduced electrogram voltage (consistent with fibrosis) in humans >50 years of age versus younger individuals.[6,7] A potential molecular basis for aging-related conduction disturbances was provided by studies showing increased phosphorylated (active) JNK (C-jun N-terminal kinase) and decreased connexin 43 expression with aging in humans, mice, and rabbits.[8] Conduction slowing and increased AF vulnerability in aged mouse hearts were reversed by a JNK inhibitor that reduced JNK phosphorylation and restored connexin 43 expression.[8] Pharmacological and genetic JNK activation downregulated atrial connexin 43 and slowed conduction. Aging has also been associated with action potential prolongation in dogs[9] and enhanced triggered activity/AF because of spontaneous intracellular calcium releases under glycolytic inhibition in rats.[10]

Novel Role for JNK in Atrial Ectopy and AF Susceptibility

Accumulating evidence points to abnormal Ca^{2+} handling in atrial ectopic activity in man,[11] which plays an important role in AF initiation.[12] In this issue of *Circulation Research*, Yan et al[13] provide thought-provoking data linking JNK to CaMKII (Ca^{2+}/calmodulin-kinase type-II) activation and AF promotion with aging. The authors use a wide range of elegant tools in human, rabbit, and mouse tissues/cells to provide compelling data in support of their thesis (Figure). Their basic biological model was the aging mouse, with comparisons between 2- to 2.5-month-old mice (equivalent to 14-year-old humans) and 24- to 32-month-old mice (60–80-year-old human equivalent). They noted increased JNK phosphorylation and AF inducibility in aged mice, along with increased Ca^{2+}-wave frequency on confocal line scanning of atrial cardiomyocytes. Both sarcoplasmic reticulum (SR) Ca^{2+} load and leak were increased, and the decay time constant of the intracellular Ca^{2+} signal was slowed, in aged mouse cardiomyocytes. JNK activation with an organic activator (anisomycin) or in mice with an inducible phosphomimetic mutation of the upstream kinase (MKK7D [mitogen activated protein kinase kinase 7]), reproduced the Ca^{2+}-handling and AF-promoting features of aging, whereas they were suppressed by a JNK inhibitor in elderly mice. The effects of anisomycin to increase Ca^{2+}-wave frequency and promote AF were not manifested in JNK2-KO (JNK knockout) mice. Anisomycin enhanced the open probability of RyR2 (ryanodine receptor 2) channels in lipid bilayers—an effect reversed by JNK inhibition or CaMKII block with KN93. These experiments demonstrate that cardiac JNK activation increases with aging, enhancing AF vulnerability and altering Ca^{2+} handling to cause diastolic Ca^{2+} leaks that lead to arrhythmogenic intracellular Ca^{2+} waves.

The ability of CaMKII inhibition to reverse the effects of JNK on Ca^{2+} release led the authors to address the links between JNK activation and that of CaMKII and to test the possibility that CaMKII might mediate the proarrhythmic effects of JNK. CaMKII hyperphosphorylation paralleled that of JNK in aged human and rabbit atrium, and CaMKII phosphorylation was normalized in aged JNK2-KO mice and in aged WT (wild type) mice after a 10-day in vivo exposure to a JNK inhibitor. Aged mice showed increased phosphorylation at CaMKII-selective sites on RyR2 and phospholamban, also seen in MKK7D mice and with anisomycin in WT (but not JNK2-KO) mice. Young mice that overexpress

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Table. Studies of Mechanisms of AF Promotion With Aging

Reference	Model	Methods	Principal Findings
Spach et al[3]	Human atrial muscle bundles	Extracellular electrogram recordings	Lateral uncoupling with age because of microfibrosis; impaired transverse conduction
Koura et al[4]	Beagles: 1–2 mo, 6–12 mo, 6–10 y	Immunochemistry, optical mapping	Reduced lateral Cx expression; conduction relatively slower laterally with aging
Sakabe et al[5]	Human in vivo EPS	EPS, electrogram analysis	Increased electrogram fractionation, refractory period with age
Roberts-Thomson et al[6]	Human in vivo EPS	Electroanatomical mapping	Increased electrogram fractionation and conduction slowing with aging
Teh et al[7]	Human in vivo EPS	PV electroanatomical mapping	Slowed PV conduction, reduced voltage, more complex electrograms
Yan et al[8]	Human samples, rabbits, JNK-activated mice; HL-1 cells	Molecular biology, optical mapping	Aging activated JNK, reduced Cx43, reduced CV; JNK downregulated Cx43
Anyukhovsky et al[9]	Dogs with electrically maintained AF	Standard microelectrode recordings	Increased APD, APD heterogeneity with aging
Ono et al[10]	Rat Langendorf-perfused hearts	Optical mapping of V_m and $[Ca_i]$	Glycolytic inhibition caused inducible AF initiation by EADs in old rats

AF indicates atrial fibrillation; APD, action potential duration; $[Ca_i]$, intracellular $[Ca^{2+}]$; CV, conduction velocity; Cx, connexin; EAD, early afterdepolarization; EPS, electrophysiological study; JNK, C-jun N-terminal kinase; PV, pulmonary vein; and V_m, transmembrane potential.

the CaMKII-inhibitory protein AC3-I (autocamtide CaMKII inhibitor) failed to show enhanced AF vulnerability or Ca^{2+} waves on treatment with the JNK activator anisomycin. Finally, the authors presented biochemical evidence for direct JNK phosphorylation of CaMKII at its activating autophosphorylation site, T286.

Novelty, Significance, and Limitations

This study is the first to demonstrate age-dependent atrial arrhythmogenic Ca^{2+}-handling abnormalities linked to JNK activation and CaMKII phosphorylation. CaMKII activation is well known to cause ectopic cardiac firing via disturbed intracellular Ca^{2+} handling,[12] but a role for CaMKII in aging-related AF associated with arrhythmogenic Ca^{2+} mishandling and activation of CaMKII by JNK, which are convincingly shown here, have not to my knowledge been addressed previously.

These findings are important because despite the fact that age is the single most important determinant of AF risk,[1,2] the mechanistic basis for this phenomenon remains incompletely understood. Although the therapeutic options for AF have improved with the development and refinement of successful ablation procedures, major gaps remain, and an improved understanding of the underlying mechanisms is needed to provide new targets for therapeutic innovation.[12]

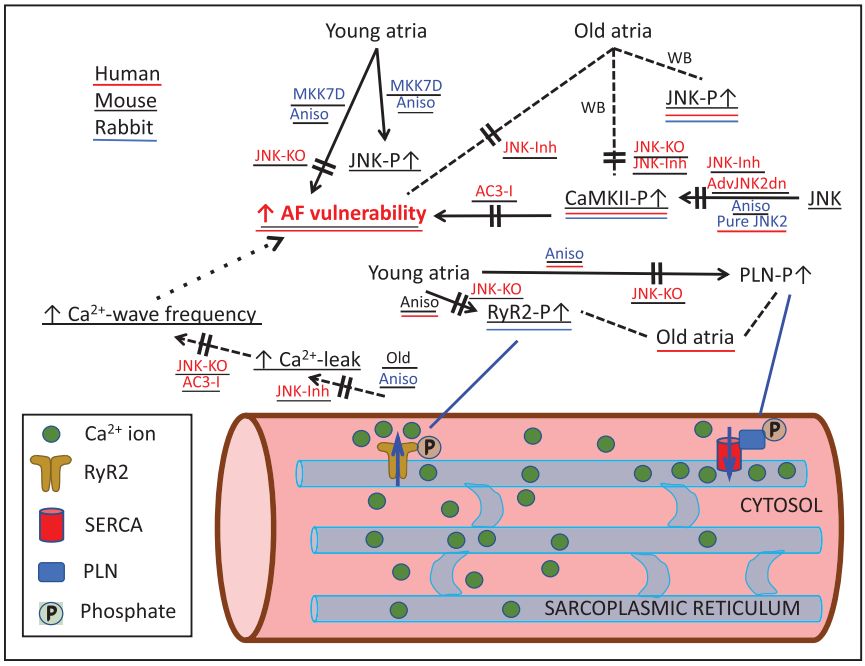

Figure. Mechanism of aging-related atrial arrhythmogenesis delineated in the study by Yan et al.[13] Biochemical mechanisms are at the (top) and the functional consequences at the (bottom). Experimental activating probes/systems are indicated in blue and blockers/inhibitors in red. Species for which observations were made are shown by color-code underline. AC3-I indicates autocamtide CaMKII inhibitor; AdvJNK2dn, JNK2 dominant-negative; AF, atrial fibrillation; Aniso, anisomycin; JNK, C-Jun N-terminal-kinase; JNK-inh, JNK inhibitor; JNK-KO, JNK knockout; MKK7D, JNK-activating phospho-mimicking mitogen-activated-kinase kinase-mutant mice; PLN, phospholamban; RYR2, ryanodine receptor 2; SERCA, SR Ca^{2+}-ATPase; and WB, Western blot.

Although the study by Yan et al describes sophisticated work using powerful techniques, some gaps remain before it can be conclusively accepted that the JNK-CaMKII-Ca^{2+} mishandling-arrhythmogenic Ca^{2+}-wave axis leads to aging-related AF. The authors did not show directly that suppressing Ca^{2+} waves prevents AF associated with aging. Further evidence could be obtained; for example, by showing that suppressing Ca^{2+} waves (eg, by buffering intracellular Ca^{2+} with a reagent like BAPTA-AM [1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid tetrakis(acetoxymethyl ester)]) suppresses AF inducibility in vitro with preparations like the aged human atrial samples that Yan et al showed are prone to AF induction. A plausible alternative mechanism for aging-related AF susceptibility is provided by previous work from this laboratory, which showed that JNK activation causes connexin 43 downregulation and conduction disturbances,[8] known to favor atrial reentry. Some details of the Ca^{2+}-mishandling phenotype remain to be clarified. For example, enhanced SR Ca^{2+} leak should reduce SR Ca^{2+} load, but the authors found the latter to be enhanced in anisomycin-treated HL-1 myocytes. They suggest that the load is enhanced because of increased SR Ca^{2+} uptake via the SR Ca^{2+}-ATPase caused by removal of the inhibitory influence of phospholamban as a result of its hyperphosphorylation. The slowed decay of the Ca^{2+} transient that they also showed, however, argues against enhanced SR-uptake enhancement that exceeds Ca^{2+} leak to produce a net increase in load.

Interestingly, there is some clinical evidence to support the notion, consistent with the central thesis of the study by Yan et al, that enhanced atrial ectopic activity may contribute importantly to aging-related AF. In an analysis of the electrophysiology-study results of 734 patients, Brembilla-Perrot et al[14] found that the susceptibility to AF induction by premature extrastimulation was actually smaller in patients >70 years of age versus younger individuals. This observation would argue against a greater risk of reentry. On the other hand, Alhede et al[15] noted that a greater recurrence rate of AF in older individuals was associated with a larger atrial ectopic-complex burden. Although these studies are far from conclusive, they are consistent with a significant role for increased atrial ectopic activity in AF promotion with aging, which the findings by Yan et al would argue is because of JNK activation and downstream phosphorylation of CaMKII, leading to arrhythmogenic Ca^{2+}-dependent triggered activity. Important questions that remain to be answered are why and how aging leads to JNK activation and whether components of this system can be targeted to provide novel therapeutic approaches to treating and preventing AF.

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References

1. Andrade J, Khairy P, Dobrev D, Nattel S. The clinical profile and pathophysiology of atrial fibrillation: relationships among clinical features, epidemiology, and mechanisms. *Circ Res*. 2014;114:1453–1468. doi: 10.1161/CIRCRESAHA.114.303211.
2. Labombarda F, Hamilton R, Shohoudi A, et al; AARCC. Increasing prevalence of atrial fibrillation and permanent atrial arrhythmias in congenital heart disease. *J Am Coll Cardiol*. 2017;70:857–865. doi: 10.1016/j.jacc.2017.06.034.
3. Spach MS, Dolber PC. Relating extracellular potentials and their derivatives to anisotropic propagation at a microscopic level in human cardiac muscle. Evidence for electrical uncoupling of side-to-side fiber connections with increasing age. *Circ Res*. 1986;58:356–371.
4. Koura T, Hara M, Takeuchi S, Ota K, Okada Y, Miyoshi S, Watanabe A, Shiraiwa K, Mitamura H, Kodama I, Ogawa S. Anisotropic conduction properties in canine atria analyzed by high-resolution optical mapping: preferential direction of conduction block changes from longitudinal to transverse with increasing age. *Circulation*. 2002;105:2092–2098.
5. Sakabe K, Fukuda N, Nada T, Shinohara H, Tamura Y, Wakatsuki T, Nishikado A, Oki T. Age-related changes in the electrophysiologic properties of the atrium in patients with no history of atrial fibrillation. *Jpn Heart J*. 2003;44:385–393.
6. Roberts-Thomson KC, Kistler PM, Sanders P, Morton JB, Haqqani HM, Stevenson I, Vohra JK, Sparks PB, Kalman JM. Fractionated atrial electrograms during sinus rhythm: relationship to age, voltage, and conduction velocity. *Heart Rhythm*. 2009;6:587–591. doi: 10.1016/j.hrthm.2009.02.023.
7. Teh AW, Kalman JM, Lee G, Medi C, Heck PM, Ling LH, Kumar S, Spence SJ, Morton JB, Kistler PM. Electroanatomic remodelling of the pulmonary veins associated with age. *Europace*. 2012;14:46–51. doi: 10.1093/europace/eur275.
8. Yan J, Kong W, Zhang Q, Beyer EC, Walcott G, Fast VG, Ai X. c-Jun N-terminal kinase activation contributes to reduced connexin43 and development of atrial arrhythmias. *Cardiovasc Res*. 2013;97:589–597. doi: 10.1093/cvr/cvs366.
9. Anyukhovsky EP, Sosunov EA, Chandra P, Rosen TS, Boyden PA, Danilo P Jr, Rosen MR. Age-associated changes in electrophysiologic remodeling: a potential contributor to initiation of atrial fibrillation. *Cardiovasc Res*. 2005;66:353–363. doi: 10.1016/j.cardiores.2004.10.033.
10. Ono N, Hayashi H, Kawase A, Lin SF, Li H, Weiss JN, Chen PS, Karagueuzian HS. Spontaneous atrial fibrillation initiated by triggered activity near the pulmonary veins in aged rats subjected to glycolytic inhibition. *Am J Physiol Heart Circ Physiol*. 2007;292:H639–H648. doi: 10.1152/ajpheart.00445.2006.
11. Voigt N, Heijman J, Wang Q, Chiang DY, Li N, Karck M, Wehrens XHT, Nattel S, Dobrev D. Cellular and molecular mechanisms of atrial arrhythmogenesis in patients with paroxysmal atrial fibrillation. *Circulation*. 2014;129:145–156. doi: 10.1161/CIRCULATIONAHA.113.006641.
12. Heijman J, Guichard JB, Dobrev D, Nattel S. Translational challenges in atrial fibrillation. *Circ Res*. 2018;122:752–773. doi: 10.1161/CIRCRESAHA.117.311081.
13. Yan J, Zhao W, Thomson JK, et al. Stress signaling JNK2 crosstalk with CaMKII underlies enhanced atrial arrhythmogenesis. *Circ Res*. 2018;122:821–835. doi: 10.1161/CIRCRESAHA.117.312536.
14. Brembilla-Perrot B, Burger G, Beurrier D, Houriez P, Nippert M, Miljoen H, Andronache M, Khaldi E, Popovic B, De La Chaise AT, Louis P. Influence of age on atrial fibrillation inducibility. *Pacing Clin Electrophysiol*. 2004;27:287–292.
15. Alhede C, Lauridsen TK, Johannessen A, Dixen U, Jensen JS, Raatikainen P, Hindricks G, Walfridsson H, Kongstad O, Pehrson S, Englund A, Hartikainen J, Hansen PS, Nielsen JC, Jons C. The impact of supraventricular ectopic complexes in different age groups and risk of recurrent atrial fibrillation after antiarrhythmic medication or catheter ablation. *Int J Cardiol*. 2018;250:122–127. doi: 10.1016/j.ijcard.2017.09.208.

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