The American Journal of Pathology, Vol. 175, No. 3, September 2009
Copyright © American Society for Investigative Pathology
DOI: 10.2353/ajpath.2009.090126

Cardiovascular, Pulmonary and Renal Pathology

Reduced Phosphoinositide 3-Kinase (p110α) Activation Increases the Susceptibility to Atrial Fibrillation

Lynette Pretorius,*† Xiao-Jun Du,*
Elizabeth A. Woodcock,* Helen Kiriazis,*
Ruby C.Y. Lin,‡ Silvana Marasco,§
Robert L. Medcalf,¶ Ziqiu Ming,*
Geoffrey A. Head,* Joon Win Tan,*
Nelly Cemerlang,* Junichi Sadoshima,‖
Tetsuo Shioi,** Seigo Izumo,**
Elena V. Lukoshkova,†† Anthony M. Dart,*§
Garry L. Jennings,* and Julie R. McMullen*

From the Baker IDI Heart and Diabetes Institute, Melbourne, Australia; the Faculty of Medicine, Nursing and Health Sciences,† and the Australian Centre for Blood Diseases,¶ Monash University, Melbourne, Australia; the Ramaciotti Centre for Gene Function Analysis,‡ University of New South Wales, Randwick Australia; the Department of Surgery,§ Alfred Hospital, Prahan, Australia; the Department of Cell Biology & Molecular Medicine,‖ University of Medicine and Dentistry of New Jersey, Newark, New Jersey; the Cardiovascular Division,** Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts; and the National Cardiology Research Centre,†† Moscow, Russia*

Atrial fibrillation (AF) is the most common sustained arrhythmia presenting at cardiology departments. A limited understanding of the molecular mechanisms responsible for the development of AF has hindered treatment strategies. The purpose of this study was to assess whether reduced activation of phosphoinositide 3-kinase (PI3K, p110α) makes the compromised heart susceptible to AF. Risk factors for AF, including aging, obesity, and diabetes, have been associated with insulin resistance that leads to depressed/defective PI3K signaling. However, to date, there has been no link between PI3K(p110α) and AF. To address this question, we crossed a cardiac-specific transgenic mouse model of dilated cardiomyopathy (DCM) with a cardiac-specific transgenic mouse expressing a dominant negative mutant of PI3K (dnPI3K; reduces PI3K activity). Adult (~4.5 months) double-transgenic (dnPI3K-DCM), single-transgenic (DCM-Tg, dnPI3K-Tg), and nontransgenic mice were subjected to morphological, functional/ECG, microarray, and biochemical analyses. dnPI3K-DCM mice developed AF and had depressed cardiac function as well as greater atrial enlargement and fibrosis than DCM-Tg mice. AF was not detected in other groups. Aged DCM-Tg mice (~15 months) with a similar phenotype to dnPI3K-DCM mice (4.5 months) did not develop AF, suggesting loss of PI3K activity directly contributed to the AF phenotype. Furthermore, increasing PI3K activity reduced atrial fibrosis and improved cardiac conduction in DCM-Tg mice. Finally, in atrial appendages from patients with AF, PI3K activation was lower compared with tissue from patients in sinus rhythm. These results suggest a link between PI3K(p110α) and AF. ***(Am J Pathol 2009, 175:998–1009; DOI: 10.2353/ajpath.2009.090126)***

Atrial fibrillation (AF) is a cardiac disorder characterized by uncoordinated atrial activation. It is the most common sustained arrhythmia presenting in cardiology departments worldwide and is associated with substantially increased mortality and morbidity from heart failure, stroke, and thromboembolism.[1] With a growing aging population the incidence of AF is increasing, adding considerably to health care costs.[1–4] The multifactorial nature of AF and a limited understanding of the molecular mechanisms responsible for the development of AF have greatly limited treatment strategies.

Supported by National Health and Medical Research Council of Australia (NHMRC) project grants 367600 (to J.R.M.) and 418935 (to E.A.W.) and program grant 225108 (to G.L.J., A.M.D., and X.J.D.). L.P., R.C.Y.L., and J.R.M. are supported by an Australian Postgraduate Award, NHMRC Peter Doherty Fellowship, and NHMRC/National Heart Foundation of Australia Career Development Award, respectively. X.J.D., E.A.W., G.A.H., and A.M.D. are NHMRC Research Fellows.

L.P. and X.J.D. contributed equally to this work.

Accepted for publication June 4, 2009.

Current address of T.S.: Kyoto University Graduate School of Medicine, Japan. Current address of S.I.: Gilead, Foster City, CA.

Address reprint requests to Dr. Julie R. McMullen, P.O. Box 6492, St. Kilda Rd. Central, Melbourne, Victoria 8008 Australia. E-mail: julie.mcmullen@bakeridi.edu.au.

Genetically modified mouse models offer a powerful approach to define molecular mechanisms. The very small size of mouse atria, together with the high heart rate (HR), make finding murine AF models difficult, as the potential for re-entry circuits is restricted. Despite these limitations, there are some genetically modified mouse models that are susceptible to AF. These include gene disruption/mutation of connexin40, KCNE1, or nuclear core component NUP155,[5–7] overexpression of RhoA,[8] basic leucine zipper inhibitor protein: JDP2,[9] angiotensin converting enzyme,[10] tumor necrosis factor α,[11] Rac1,[12] and MURC.[13]

AF often occurs in combination with heart failure, although the factors that precipitate the onset of AF in patients with (or without) pre-existing heart disease remain unclear.[14,15] We previously demonstrated that phosphoinositide 3-kinase (PI3K, p110α) is a critical regulator of adaptive physiological heart growth,[16–18] and that inhibiting PI3K(p110α) accelerates heart failure in a setting of dilated cardiomyopathy (DCM).[19] However, due to the severity of the disease progression in this mouse model (average life span of approximately 40 days[19]) it was not possible to perform functional or electrocardiogram (ECG) analyses. The rationale for hypothesizing a link between AF and reduced PI3K(p110α) activity came from multiple lines of evidence. First, we previously demonstrated that decreasing PI3K activation alters gene expression of ion channels in ventricular tissue. Second, several classes of drugs have been reported to induce AF in patients.[21] The mechanisms considered responsible include adrenergic stimulation and cardiotoxicity.[21] PI3K(p110α) is a cardioprotective protein that has been shown to inhibit pathological signaling cascades downstream of G protein coupled receptors,[19] thus loss of PI3K(p110α) would be expected to increase the likelihood of cardiotoxicity and activation of signaling proteins downstream of G protein coupled receptors. Third, we previously reported that heat shock protein 70 (Hsp70) expression is elevated in hearts of mice with increased PI3K(p110α) activity and decreased in hearts of mice with decreased PI3K(p110α) activity.[20] A number of reports have linked Hsp70 to AF. Patients with high Hsp70 expression levels have a lower incidence of postoperative AF, and an M439T substitution in Hsp70 was associated with an increased risk of postoperative AF.[22–25] Finally, advanced age and possibly obesity and diabetes are risk factors for the development of AF.[26–28] These factors are typically associated with reduced physical activity and insulin resistance. Since PI3K(p110α) is activated in the heart in response to exercise,[29] and is a critical molecular signal for insulin, one would predict that PI3K(p110α) activity is generally lower in hearts of obese patients, diabetics, and the elderly.

It was also of interest to examine electrical activity in the heart under conditions of reduced PI3K(p110α) activity because of the recent enthusiasm surrounding the development of PI3K(p110α) inhibitors as anticancer agents.[30,31] Uncontrolled activation of the PI3K(p110α) pathway is a critical molecular mechanism by which cancer cells bypass normal growth-limiting controls. However, a challenge in targeting PI3K(p110α) relates to its diverse actions in numerous cell types.[32] As noted earlier, PI3K(p110α) is a critical regulator of adaptive physiological heart growth[16–18] and we recently demonstrated that inhibiting PI3K(p110α) accelerates heart failure in the compromised heart.[19]

To reduce PI3K(p110α) activity in a setting of cardiac stress, we genetically crossed a cardiac-specific transgenic (Tg) mouse model of DCM,[33] with a cardiac-specific transgenic mouse expressing a dominant negative mutant of the p110α isoform of PI3K (dnPI3K).[16] Expression of the dnPI3K transgene reduces PI3K activity in cardiac myocytes by approximately 77%.[16] We report here that reduced PI3K(p110α) activity increases the susceptibility to AF in a mouse model of DCM and that PI3K activity is also reduced in atrial appendages from patients with AF compared with those in sinus rhythm.

Materials and Methods

Animals

Animal care and experimentation were approved by the Alfred Medical Research and Education Precinct Animal Ethics Committee.

Generation of Mice with DCM and Reduced PI3K Activity

Cardiac-specific DCM-Tg (mammalian sterile 20-like kinase 1, line no. 28; C57BL/6 background) and cardiac-specific dnPI3K-Tg (FVB/N; provided by P. Kang, BIDMC) were generated and genotyped as described.[16,33] The DCM-Tg model has been considered clinically relevant because mice develop DCM as a consequence of increased apoptosis.[33] Male heterozygous DCM-Tg and female heterozygous dnPI3K-Tg were crossed to generate 1) double transgenic mice: dnPI3K-DCM, expressing both transgenes ($dnPI3K^{+/-}$ $DCM^{+/-}$), 2) dnPI3K-Tg ($dnPI3K^{+/-}$ $DCM^{-/-}$), 3) DCM-Tg ($dnPI3K^{-/-}$ $DCM^{+/-}$) and 4) nontransgenic littermate controls (NTg; $dnPI3K^{-/-}$ $DCM^{-/-}$). This study is focused on the above four genotypes. However, a small subset of DCM mice with increased PI3K activity were also generated (described below) to assess whether increasing PI3K activity could reverse some of the phenotypic characteristics of the DCM model. All mice were bred on the same genetic background (C57BL/6-FVB/N).

Generation of Mice with DCM and Increased PI3K Activity

Male heterozygous DCM-Tg and female heterozygous constitutively active (ca) PI3K-Tg (caPI3K; PI3K activity increased by 6.5-fold in the heart[16]) were crossed to generate caPI3K-DCM expressing both transgenes ($caPI3K^{+/-}$ $DCM^{+/-}$).

Transthoracic Echocardiography

In vivo heart function and chamber dimensions were assessed by echocardiography using a Hewlett-Packard Sonos 5500 ultrasonograph with a 15-MHz linear array

transducer. Mice were anesthetized with 2,2,2-tribromoethanol (240 mg/kg, i.p.). Left ventricular (LV) wall thicknesses [LV posterior wall (LVPW) and interventricular septum (IVS)], LV chamber dimensions (LVD) at end-diastole and end-systole (LVDd and LVDs), and fractional shortening [FS = (LVDd-LVDs)/LVDd] were determined from M-mode images. Left atrial size was determined from long-axis two-dimensional images at end-systole.[34]

Micromanometry

Arterial pressures, LV systolic and end-diastolic pressures (LVSP, LVEDP), maximal rates of rise and fall of LV pressures (dP/dt_{max}, dP/dt_{min}) and HR were measured in anesthetized mice (ketamine/xylazine/atropine 100/10/1.2 mg/kg i.p.) using a 1.4-Fr Millar (Houston, TX) MIKRO-TIP catheter and a Powerlab system (ADInstruments, Sydney, Australia).[35]

Surface, Intracardiac, and Telemetry ECG Recordings

Surface

ECG recordings were measured in anesthetized mice (2,2,2-tribromoethanol: 240 mg/kg, i.p.) using the Powerlab system and BioAmp (ADInstruments). Two pairs of 27-gauge needle electrodes were placed subcutaneously and recordings were made from a chest lead (equivalent to V5). All signals were sampled at 1 kHz for a period of 5 to 10 minutes. Averaged HRs, P-R intervals, R-R intervals, QRS intervals and amplitudes of positive R- and P-waves were measured digitally using ADInstruments (Chart 5 Pro ECG analysis module).

Intracardiac ECG

An electrophysiology catheter (EPR-800, 1.1F; Millar) was inserted into the jugular vein for placement inside the right atrium and right ventricle of anesthetized mice (ketamine/xylazine/atropine, 100/10/1.2 mg/kg i.p.). Surface ECG recordings (lead II position) were measured simultaneously.

Telemetry

To confirm anesthetized ECG findings, ambulatory ECGs were recorded in conscious unrestrained mice after implantation of telemeters (TA10EA-F20, Data Sciences International) with leads subcutaneously positioned at the right foreleg and left side of the chest (ie, similar position to surface leads; V5). A 7-day recovery period was allowed before recording for 24 hours. Files were recorded using ART acquisition (Data Sciences International, Minneapolis, MN) or Labview data acquisition.[36]

Morphometry and Histopathology

Body weight, tibia length, and wet weights of ventricles, atria, and lungs were measured. Fibrosis was quantified in ventricular and atrial sections (6 μm) stained with Masson's trichrome using Olympus Image Pro Plus 6 (Media Cybergenetics, Bethesda, MD).[20]

Fibrin Zymography

The degree of fibrinolytic activity in mouse plasma was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10% gels) followed by fibrin zymography[37,38] using recombinant human t-PA protein as a standard. Five microliters of undiluted mouse plasma was loaded per lane. Gels were incubated at 37°C in a humidified chamber until regions of proteolysis appeared.

Gelatin Zymography

Matrix metalloproteinase 2 (MMP-2) and MMP-9 activities in protein from mouse ventricular tissue were assessed by gelatin zymography as described.[39]

PI3K Activity in Mouse Ventricular Tissue and Human Atrial Tissue

PI3K activity was assessed in mouse ventricular tissue and human atrial tissue.[16,20] Mouse ventricular tissue or human atrial tissue lysate (1 mg) was immunoprecipitated with an anti-p85 antibody (0.5 μl, Upstate Biotechnology, New York, NY) and subjected to an *in vitro* lipid kinase assay using phosphatidylinositol as a substrate. Part of the immunoprecipitated enzyme was subjected to Western blotting and probed with the anti-p85 antibody to confirm that an equal amount of enzyme was used for the assay.

The study of human atrial tissue was approved by the Alfred Hospital Human Ethics committee. Tissue samples of atrial appendages were collected from patients undergoing coronary artery bypass graft (CABG) surgery (right appendage only) who did or did not develop acute AF, as well as patients undergoing mitral valve surgery (left appendages) with chronic AF. Medications (statins, β-blockers, and ACE inhibitors) were evenly distributed between groups and stopped the night before surgery. Patients with diabetes were not included in the study.

Western Blotting

Phosphorylation of Akt and extracellular signal regulated kinase (ERK) 1/2 were assessed in mouse ventricular tissue as described.[16]

Gene Expression

Northern Blotting

Sarcoplasmic reticulum Ca^{2+} ATPase 2a (SERCA2a) and GAPDH were assessed in mouse ventricular samples as described.[40]

Table 1. Cardiac Dimensions, Function and Hemodynamics, and Organ Weights in Female NTg, dnPI3K-Tg, DCM-Tg, and dnPI3K-DCM at 4.5–4.9 Months of Age

	NTg	dnPI3K	DCM	dnPI3K-DCM
Echocardiography	$N = 6$	$N = 5$	$N = 6$	$N = 6$
Heart rate (beats/min)	509 ± 23	500 ± 16	476 ± 22	584 ± 28
LVDd (mm)	3.93 ± 0.12	4.01 ± 0.10	4.48 ± 0.20*†	5.17 ± 0.14*†‡
LVDs (mm)	1.95 ± 0.11	2.03 ± 0.10	2.86 ± 0.17*†	3.92 ± 0.26*†‡
LVPW (mm)	0.71 ± 0.03	0.58 ± 0.05*	0.60 ± 0.04	0.39 ± 0.05*†‡
IVS (mm)	0.76 ± 0.03	0.63 ± 0.08	0.55 ± 0.03*	0.36 ± 0.04*†‡
FS (%)	51 ± 2	49 ± 2	36 ± 2*†	24 ± 4*†‡
Left atrial size (mm^2)	4.0 ± 0.3	2.6 ± 0.3	8.1 ± 0.9*†	13.8 ± 1.7*†‡
Catheterization	$N = 5$	$N = 3$	$N = 4$	$N = 4$
HR (beats/min)	352 ± 19	360 ± 52	382 ± 18	379 ± 18
SBP (mmHg)	117 ± 5	100 ± 3*	84 ± 4*†	76 ± 5*†
DBP (mmHg)	80 ± 3	75 ± 4	61 ± 3*†	57 ± 2*†
LVSP (mmHg)	110 ± 4	101 ± 3	81 ± 2*†	74 ± 3*†
LVEDP (mmHg)	6 ± 1	6 ± 0	11 ± 1*†	15 ± 0*†‡
dP/dt_{max} (mmHg/s)	7427 ± 383	7064 ± 391	6722 ± 645	4128 ± 245*†‡
dP/dt_{min} (mmHg/s)	7039 ± 411	6105 ± 245	5011 ± 388*	3259 ± 85*†‡
Morphometrics	$N = 7–8$	$N = 7$	$N = 8–9$	$N = 5–6$
Body weight (BW, g)	25.3 ± 0.6	26.9 ± 0.7	27.2 ± 0.7	27.8 ± 0.7
Ventricular weight (mg)	105.6 ± 4.5	87.0 ± 1.8*	134.6 ± 5.4*†	138.5 ± 11.1*†
Ventricular/BW (mg/g)	4.17 ± 0.12	3.24 ± 0.07*	4.89 ± 0.14*†	4.92 ± 0.38*†
Ventricular/TL (mg/mm)	6.13 ± 0.24	5.03 ± 0.07*	7.69 ± 0.31*†	7.82 ± 0.64*†
Atria weight (mg)	6.0 ± 0.2	4.4 ± 0.3	16.4 ± 2.1*†	32.8 ± 8.2*†‡
Lung weight (mg)	148.6 ± 2.5	142.4 ± 2.0	190.4 ± 7.1*†	226.7 ± 11.5*†‡

LVDd, LVDs, LV dimension at diastole or systole; LVPW, LV posterior wall thickness; IVS, interventricular septum thickness; FS, fractional shortening; SBP, DBP, systolic or diastolic blood pressure; LVSP, LV systolic pressure; LVEDP, LV end-diastolic pressure; dP/dt_{max} and dP/dt_{min}, maximum rate of rise and decay of LV pressures; TL, tibial length.

*$P < 0.05$ versus NTg.

†$P < 0.05$ versus dnPI3K.

‡$P < 0.05$ versus DCM.

Microarray

Gene expression profiling was performed on 32 mouse atria samples using Affymetrix GeneChip mouse gene 1.0 ST arrays. RNA was extracted using TRIzol (Invitrogen) and sense DNA targets were generated from 100 ng of total RNA according to the manufacturer's protocol (Affymetrix, Santa Clara, CA). Hybridization, wash, and scan were performed at the Ramaciotti Centre for Gene Function Analysis (University of New South Wales, Australia). Array data were processed using robust multiple-array average (RMA) normalization (Partek v6.4, Partek Inc. St. Louis, Missouri). Principle component analysis was used to identify batch and technical variations and these were removed for subsequent analysis. Data were analyzed accordingly. Differences across genotypes were compared using analysis of variance and unpaired *t*-test to look for differences between specific genotypes. Since left and right atria from the same mouse were processed, a "matched-paired-organ" analysis was performed using the ratio of left/right atria of the same mouse. Analysis of variance was then performed to look for differential gene expression between genotypes. This analysis is considered more statistically robust to identify genes of interest.

Gene lists were generated and the q value at 0.01 level was calculated for FDR. Hierarchical clustering and functional annotation were performed. The experimental design, RNA extraction and microarray experiment in this study are all MIAME (minimum information about a microarray experiment)-compliant. The complete raw and normalized array data are available through the Gene Expression Omnibus of the National Centre for Biotechnology Information (*http://www.ncbi.nlm.nih.gov/geo/*, accession number GSE12420). In this study we have presented gene expression profiles of potassium and metabolism genes because of previous links with AF.[41–43]

Statistics

Results are presented as mean ± SE. Differences between groups were compared using one-way analysis of variance followed by the Fisher's protected least significant difference post hoc test (unless otherwise shown). A value of $P < 0.05$ was considered significant.

Results

Biochemical, Functional, and Morphological Characteristics of Mice with Reduced PI3K Activity on a Setting of DCM (dnPI3K-DCM)

The phenotypes of the single transgenics (ie, dnPI3K-Tg and DCM-Tg) have previously been described.[16,33] In brief, the reduction in cardiac PI3K activity in dnPI3K-Tg is associated with a reduction in heart weight of ~20% (see ventricular weight/body weight, Table 1) but cardiac function is normal under basal conditions (Table 1). DCM-Tg develop DCM as a consequence of overexpression of a stress-activated protein kinase (mammalian sterile 20-like kinase 1[33]). This model is associated with increased ventricular chamber dimensions (LVDd and

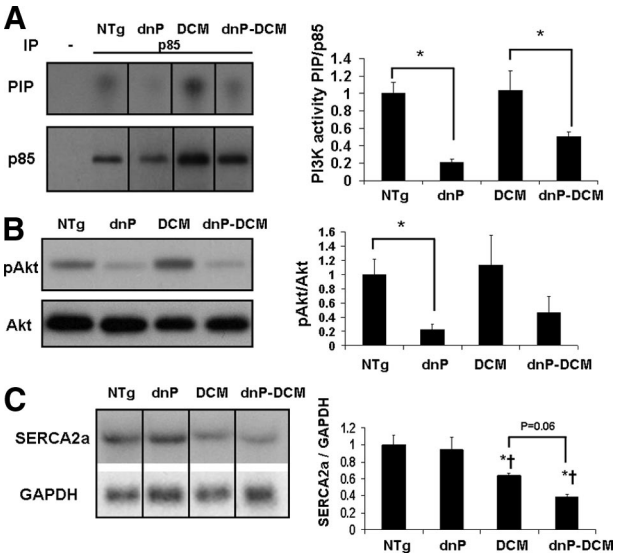

Figure 1. PI3K activity, pAkt, and SERCA2a gene expression in mouse ventricular samples from NTg, dnPI3K (dnP), DCM, and dnPI3K-DCM (dnP-DCM). **A:** PI3K activity (**left, upper panel**). IP, immunoprecipitate; −, negative control (sample without antibody); PIP, phosphatidylinositol 3-phosphate. A portion of the immunoprecipitated antibody was subjected to Western blotting and probed with an anti-p85 antibody (**left, lower panel**). Quantitative analysis of PI3K activity relative to p85 (**right panel**). NTg: $N = 6$, dnP: $N = 6$, DCM: $N = 8$, dnPI3K-DCM: $N = 7$. **B:** Representative Western blot showing pAkt and total Akt (left panel). Quantitative analysis of pAkt relative to total Akt (**right panel**). NTg: $N = 3$ in each group. $^{*}P < 0.05$ (unpaired *t*-test). **C:** Representative Northern blot showing SERCA2a gene expression (**left, upper panel**) relative to GAPDH (**left, lower panel**). Quantitative analysis (right panel). NTg: $N = 6$, dnP: $N = 5$, DCM: $N = 6$, dnPI3K-DCM: $N = 6$. $^{*}P < 0.05$ versus NTg, $^{\dagger}P < 0.05$ versus dnP. NTg was normalized to 1.0 in each panel.

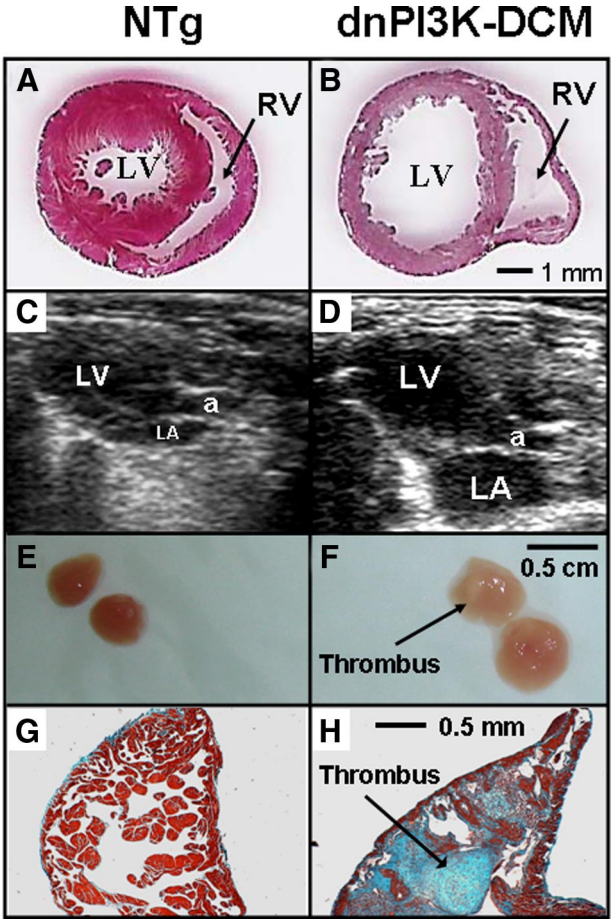

Figure 2. Pathological findings in dnPI3K-DCM. Cardiac transverse sections from NTg (**A**) and dnPI3K-DCM (**B**). Enlarged atrial size in dnPI3K-DCM versus NTg by echocardiography (**C** and **D**) and at autopsy (**E** and **F**). LA, left atrium; LV, left ventricle; RV, right ventricle; a, aorta. Microscopic view showing fibrosis (blue staining Masson's trichrome) and organic thrombus (**arrow**) in the atria of dnPI3K-DCM versus NTg (**G** and **H**).

LVDs, Table 1), thinning of ventricular walls (LVPW and IVS, Table 1) and depressed systolic function (FS, Table 1). DCM-Tg also have enlarged atria and congested lungs compared with NTg (Table 1).

As previously reported,[16] PI3K activity and the phosphorylation of a downstream target, pAkt, was significantly lower in ventricular tissue of dnPI3K transgenics compared with NTg (Figure 1, A and B). PI3K activity was also significantly depressed in dnPI3K-DCM compared with DCM and a similar pattern (although not significant) was observed for pAkt/total Akt (Figure 1, A and B). The phosphorylation of pERK1/2 relative to total ERK1/2 was not different between groups (data not shown).

The heart failure phenotype of dnPI3K-DCM was more severe than DCM-Tg alone [lifespan reduced from 8.3 ± 0.3 months ($n = 5$) to 4.3 ± 0.6 months ($n = 7$, $P < 0.05$)]. At ~4.5 months of age, dnPI3K-DCM showed signs of severe DCM (Figure 2, A and B). By echocardiography, dnPI3K-DCM had dilated LV chambers, thin ventricular walls, enlarged atria, and depressed cardiac function (Table 1; Figure 2, C and D). The presence of DCM and heart failure was also indicated by a reduction in LV contractile parameters including dP/dt_{max}, dP/dt_{min} and LVSP, as well as an increase in LVEDP (Table 1). Consistent with the more severe heart failure phenotype in dnPI3K-DCM compared with DCM, normalized SERCA2a expression (a critical determinant of Ca^{2+} homeostasis and cardiac contractility) tended to be lower ($P = 0.06$) in hearts of dnPI3K-DCM than DCM (Figure 1C), and was significantly lower than NTg and dnPI3K.

At autopsy, dnPI3K-DCM had dilated hearts and enlarged atria, and chronic thrombi were present in the left atrium in ~50% of mice (Figure 2, E and F). Total ventricular weights normalized for body or tibial length were increased by approximately 20 to 25% in dnPI3K-DCM compared with NTg (Table 1). The atrial weight of dnPI3K-DCM was fivefold greater than NTg and twofold greater than DCM-Tg. dnPI3K-DCM also had elevated lung weights, indicative of lung congestion (Table 1).

Atrial thrombus formation is a common complication of AF that is associated with an increased risk of stroke. Thrombus formation in AF can be related to several underlying pathophysiological mechanisms including anatomical and structural changes, abnormal changes in blood constituents, or atrial stasis due to heart failure. In the current study, global fibrinolytic activity present in mouse plasma was not different between the four groups of mice as assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fibrin zymography (Figure 3A). However, atrial fibrosis was 5.5-fold and 2.2-fold higher in dnPI3K-DCM and DCM-Tg, respectively, com-

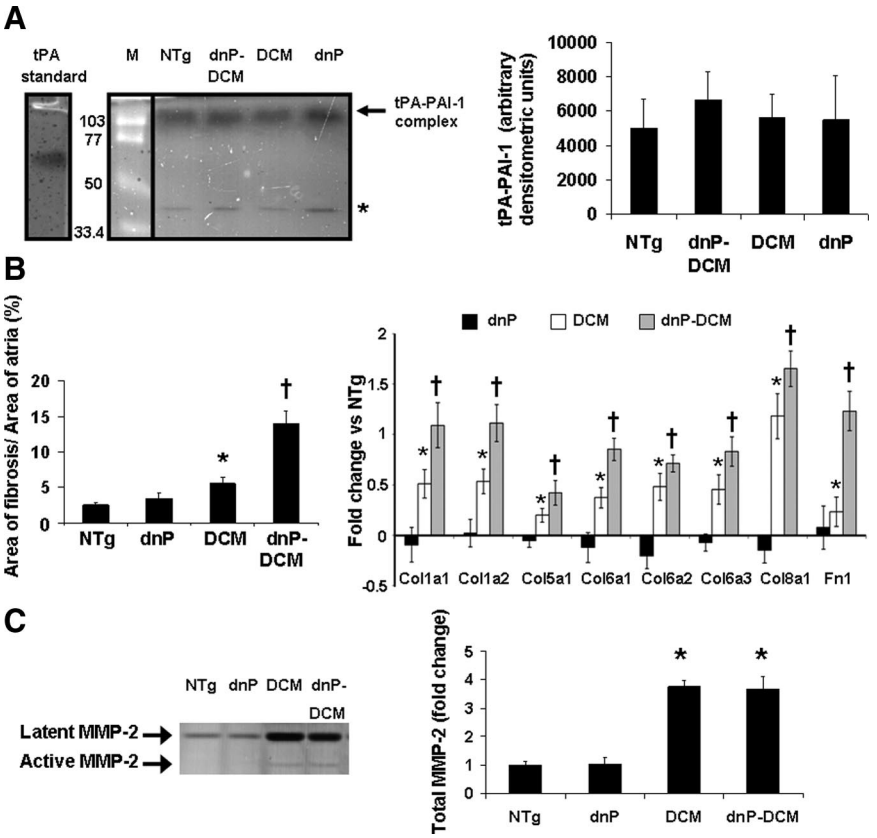

Figure 3. Pathophysiological mechanisms responsible for atrial thrombus formation in dnPI3K-DCM. **A:** Global fibrinolytic activity in mouse plasma of NTg, dnP-DCM (dnPI3K-DCM), DCM, and dnP (dnPI3K). Fibrin zymography (**left** panel): Tissue plasminogen (tPA) standard; molecular weight marker (M) in kilodaltons; tPA-plasminogen activator inhibitor 1(PAI-1) complex. Quantitative analysis of the tPA-PAI-1 complex (**right** panel). The fibrinolytic activity that migrates below the 50-kd molecular weight marker (marked with an **asterisk**) may possibly represent mouse urokinase (u-PA), which migrates at 45–48 kd. Regardless of its formal identity, there was no obvious difference in the level of this particular fibrinolytic moiety between the groups. $N = 4$ in each group. **B:** Atrial fibrosis in NTg, dnPI3K-Tg, DCM-Tg, and dnPI3K-DCM. Quantitative analysis of area of fibrosis relative to area of the atria (**left** panel). NTg: $N = 6$, dnP: $N = 4$, DCM: $N = 6$, dnPI3K-DCM: $N = 4$. Gene expression changes measured by microarray of extracellular matrix- and fibrosis-related genes. Col, procollagen types; Fn1, fibronectin (**right** panel). $^*P < 0.05$ versus NTg and dnPI3K; $^\dagger P < 0.05$ versus DCM. $N = 4$ in each group. **C:** MMP-2 activity (gelatin zymography) in ventricular tissue. NTg was normalized to 1.0. $^*P < 0.05$ versus NTg and dnPI3K. $N = 3$ in each group.

pared with NTg (Figure 2, G and H; Figure 3B). Consistent with this finding, gene expression changes of a number of extracellular matrix- and fibrosis-related genes were higher in DCM-Tg and dnPI3K-DCM compared with NTg and dnPI3K-Tg (Figure 3B). Ventricular collagen deposition was also significantly greater in dnPI3K-DCM (4.45 ± 0.43%, $N = 6$) than DCM-Tg (3.42 ± 0.34%, $N = 3$) and NTg (1.26 ± 0.04%, $N = 6$). To explore the possibility that changes in components of the blood may activate pro-MMPs to MMPs and contribute to atrial and LV structural disarrangement with increased deposition of collagen, we assessed latent and active forms of two MMPs in ventricular tissue (inadequate protein available from mouse atria) that have been associated with marked structural abnormalities in the atria in a setting of heart failure ie, MMP2 and MMP9.[44,45] By gelatin zymography we found no changes in latent or active MMP9 (data not shown). However, both latent and active MMP2 were increased in ventricles of DCM and dnPI3K-DCM compared with NTg and dnPI3K (Figure 3C), although there were no differences between DCM and dnPI3K-DCM. Together, these data suggest that the incidence of atrial thrombus in dnPI3K-DCM is the consequence of heart failure and AF per se, as opposed to changes in blood constituents causing AF.

dnPI3K-DCM Develop Atrial-Ventricular Conduction Blockade (AVB) and AF

Mild to major ECG abnormalities were observed in dnPI3K-Tg, DCM-Tg, and dnPI3K-DCM compared with NTg (Table 2; Figure 4, A–D). dnPI3K-Tg had slightly blunted R- and P- amplitudes (Table 2). This may be due to the reduced ventricular mass of dnPI3K-Tg (Table 1). DCM-Tg had prolonged P-R intervals and a smaller

Table 2. ECG Analyses from NTg, dnPI3K-Tg, DCM-Tg, and dnPI3K-DCM at 4.2–4.9 Months of Age

Parameter	NTg ($N = 12$)	dnPI3K ($N = 8$)	DCM ($N = 6$)	dnPI3K-DCM ($N = 8$)
HR (beats/min)	477 ± 7	475 ± 11	472 ± 20	514 ± 18
R-R (ms)	126 ± 2	127 ± 3	128 ± 5	118 ± 4
P-R (ms)	37 ± 1	39 ± 1	53 ± 2*†	86 ± 9*†‡
QRS (ms)	9.0 ± 0.2	9.0 ± 0.4	8.0 ± 0.2	8.0 ± 0.3
P-amplitude (mV)	0.14 ± 0.01	0.08 ± 0.01*	0.09 ± 0.01*	0.05 ± 0.01*†‡
R-amplitude (mV)	1.55 ± 0.08	1.31 ± 0.09*	0.93 ± 0.09*†	0.40 ± 0.08*†‡

*$P < 0.05$ versus NTg.
†$P < 0.05$ versus dnPI3K.
‡$P < 0.05$ versus DCM.

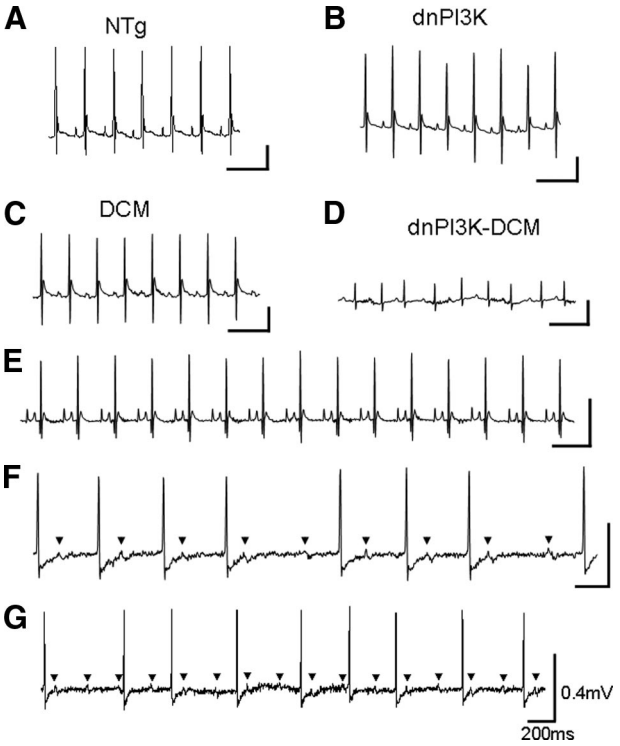

Figure 4. Representative ECGs from NTg (**A**), dnPI3K-Tg (**B**), DCM-Tg (**C**), and dnPI3K-DCM (**D**). dnPI3K-DCM displayed various degrees of AVB. **E:** First-degree AVB (constant prolonged PR); **F:** Mobitz type 1 second-degree AVB (gradual prolongation of the P-R intervals before complete block, Wenckebach phenomenon; solid triangles indicate P-waves); **G:** Third-degree AVB (no relationship between P waves and QRS complexes).

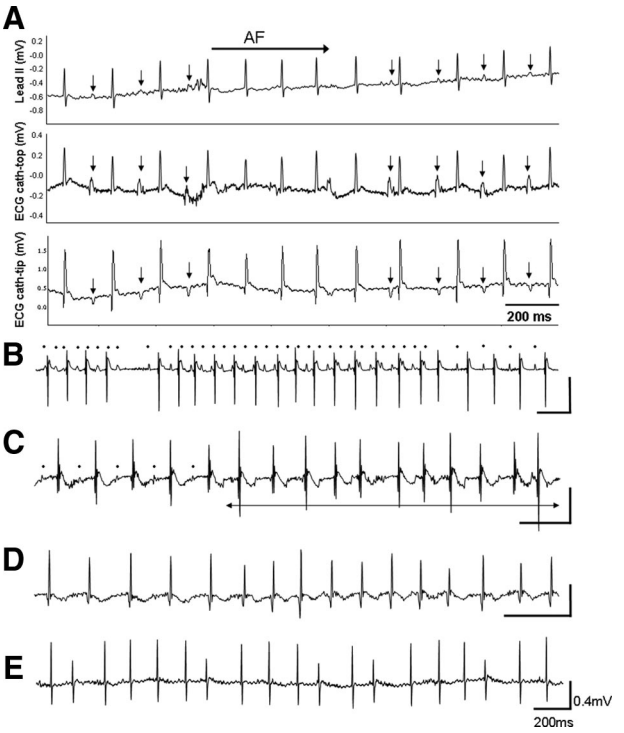

Figure 5. ECGs from dnPI3K-DCM showing episodes of AF. **A:** Short episode of AF. Intracardiac ECG using an electrophysiology catheter; P-waves are indicated with **arrows**. Simultaneous recordings from lead II (**upper panel**) and intracardiac catheter (**lower two panels**). **B:** Atrial tachycardia (dots indicate P-waves), atrial rate ~1000 beats/min. **C:** Short episode of AF (indicated by **horizontal arrow**). **D** and **E:** Prolonged episode of AF (minutes), demonstrated by absence of P-waves and irregular R-R intervals.

R-amplitude than both dnPI3K-Tg and NTg (Table 2). All dnPI3K-DCM had markedly suppressed P- and R-amplitudes compared with NTg (Table 2; Figure 4, A and D). dnPI3K-DCM had P-waves with aberrant morphologies including double peaks (Figure 4E). All dnPI3K-DCM had prolonged P-R intervals and displayed first degree (Figure 4E), second degree (Figure 4F), and third degree block (Figure 4G). Finally, despite recording ECG signals in anesthetized mice for only short periods of time (5–10 minutes), AF occurred spontaneously in ~40% of dnPI3K-DCM (six of 16 animals, Figure 5E). AF was considered if P-waves were absent during periods with overtly irregular R-R intervals. The absence of P-waves was confirmed by intracardiac catheter recordings (Figure 5A). Such episodes were not observed in NTg, dnPI3K, or DCM-Tg ($n = 6–12$). Episodes of atrial tachycardia and AF (seconds to minutes) were also apparent in conscious dnPI3K-DCM by ambulatory ECG (Figure 5, B–D). ECG telemetry studies suggest that the incidence of AF is higher than 40% when mice are monitored for 24-hour periods; by telemetry AF was detected in six of six dnPI3K-DCM.

ECG Characteristics of Aged DCM-Tg and dnPI3K-Tg

ECG measurements were recorded in aged DCM-Tg to examine whether AF in dnPI3K-DCM was simply a result of a more severe phenotype in dnPI3K-DCM compared with DCM-Tg. DCM-Tg have an average lifespan of ~8 months although a small percentage of mice live considerably longer. Aged DCM-Tg (15.0 ± 1.3 months, $n = 6$) displayed a similar heart failure phenotype to that of dnPI3K-DCM [~4.5 months; similar atrial weights (DCM: 33.8 ± 3.9; $n = 5$ versus dnPI3K-DCM: 32.8 ± 8.2 mg; Table 1) and lung congestion (lung weights-DCM: 285.5 ± 47.3; $n = 6$ versus dnPI3K-DCM: 226.7 ± 11.5 mg, Table 1)]. However, ECG abnormalities remained more severe in dnPI3K-DCM (Table 2) than aged DCM-Tg (Table 3). The R-amplitude of aged DCM-Tg was reduced ($P < 0.05$) compared with younger DCM-Tg but remained greater than that observed in dnPI3K-DCM

Table 3. Parameters Derived from ECGs from Aged NTg, dnPI3K, and DCM-Tg

Parameter	NTg ($N = 6$)	dnPI3K ($N = 5$)	DCM ($N = 6$)
Age (months)	13.5 ± 2.7	16.1 ± 1.6	15.0 ± 1.3
HR (beats/min)	491 ± 21	485 ± 16	430 ± 53
R-R (ms)	124 ± 6	124 ± 4	159 ± 33
P-R (ms)	37 ± 1	44 ± 2*	63 ± 7†‡
QRS (ms)	10.0 ± 0.0	11.0 ± 0.1	11.0 ± 0.1
P-amplitude (mV)	0.18 ± 0.02	0.09 ± 0.01†	0.09 ± 0.02†
R-amplitude (mV)	1.48 ± 0.07	1.01 ± 0.18†	0.52 ± 0.06†‡

*$P < 0.05$ versus NTg (unpaired *t*-test).
†$P < 0.05$ versus NTg (ANOVA).
‡$P < 0.05$ versus dnPI3K (ANOVA).

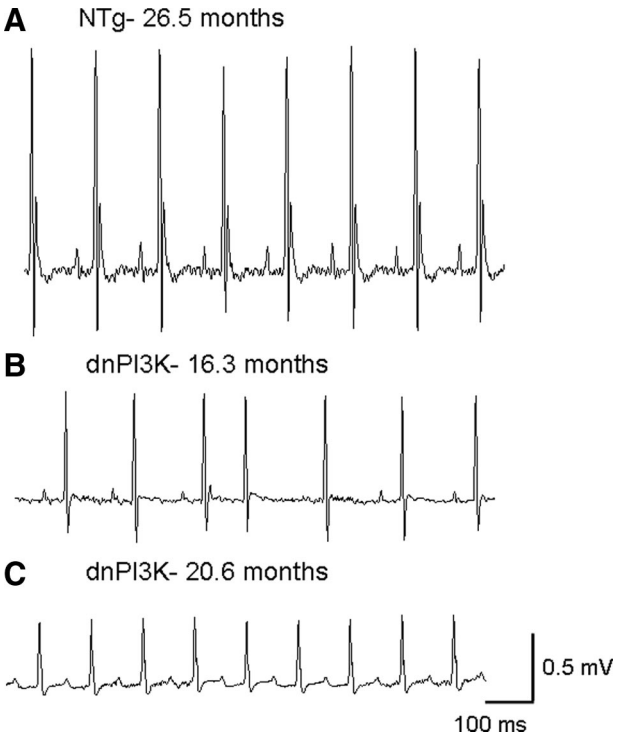

Figure 6. Aged dnPI3K-Tg have blunted R-amplitudes, prolonged P-R intervals, and periods with irregular R-R intervals. ECGs from an aged NTg (**A**) and aged dnPI3K-Tg (**B** and **C**).

(Tables 2 and 3). The P-R interval was not significantly different between aged and younger DCM-Tg, and AF was not observed in aged DCM-Tg. Interestingly, in aged dnPI3K-Tg (16.1 ± 1.6 months) there was a small but significant increase in P-R interval compared with younger dnPI3K-Tg (~4.5 months, $P < 0.05$, Table 2) and aged NTg (Table 3). The R-amplitude of aged dnPI3K-Tg was smaller than aged NTg, and aged dnPI3K-Tg displayed periods with irregular R-R intervals (Figure 6, A–C; Table 3).

Gene Expression Profiles in Mouse Atrial Samples

Next we assessed whether our AF mouse model (dnPI3K-DCM) was associated with changes in gene expression that have been reported in humans or large animal AF models. The dnPI3K transgene alone (decrease in PI3K activity) had a significant effect on the gene expression of potassium channels and genes involved in metabolic pathways compared with atrial samples from NTg (Figure 7, A and B). Gene expression fold changes versus NTg were larger again in DCM and greatest in dnPI3K-DCM (Figure 7, A and B).

Reduced PI3K Activity in Human Atrial Appendages from Patients with AF

To examine whether changes in PI3K activation may be a contributing factor to the development of AF in humans, we assessed PI3K activity in atrial appendages from

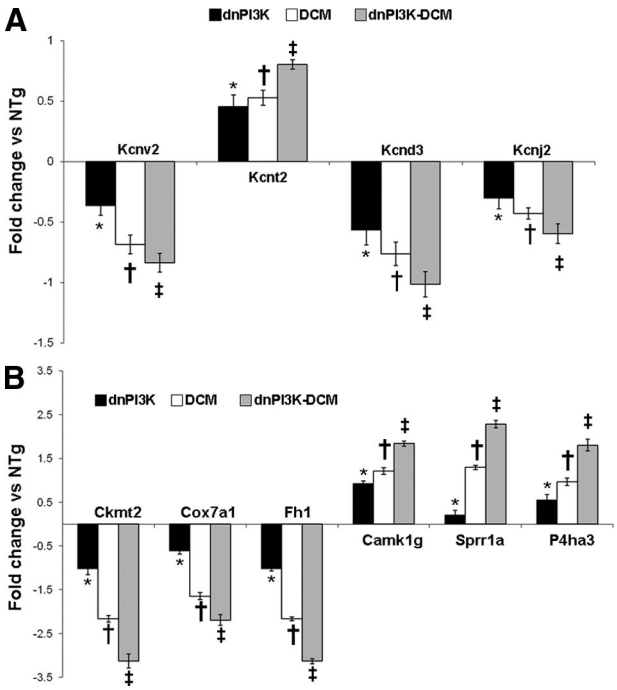

Figure 7. Gene expression changes measured by microarray of potassium channels (**A**), and metabolism genes (**B**). $^{*}P < 0.05$ versus NTg, $^{\dagger}P < 0.05$ versus NTg and dnPI3K. $^{\ddagger}P < 0.05$ versus NTg, dnPI3K and DCM. $N = 4$ in each group. Potassium channels–Kcnv2: potassium channel, subfamily V, member 2; Kcnt2: potassium channel, subfamily T, member 2; Kcnd3: potassium voltage-gated channel, Shal-related family, member 3; Kcnj2: potassium inwardly rectifying channel, subfamily J, member 2. Metabolism genes–Ckmt2: creatine kinase, mitochondrial 2; Cox7a1: cytochrome *c* oxidase, subunit VIIa 1; Fh1: fumarate hydratase 1; Camk1g: calcium/calmodulin-dependent protein kinase I γ; Sprr1a: small proline-rich protein 1A; P4ha3: procollagen-proline, 2-oxoglutarate 4-dioxygenase.

patients with acute or chronic AF. PI3K(p110α) activity was measured in atrial appendages from patients undergoing CABG surgery who did [66 ± 3 years, $n = 4$ (three females, one male)] or did not [64 ± 4 years, $n = 6$ (five males, one female)] develop AF postoperatively, and in atrial appendages from patients undergoing mitral valve surgery with chronic AF [76 ± 3 years, $n = 5$ (two males, three females)]. PI3K activity was lower in atrial samples from patients with acute or chronic AF compared with CABG patients without AF (Figure 8, A and B).

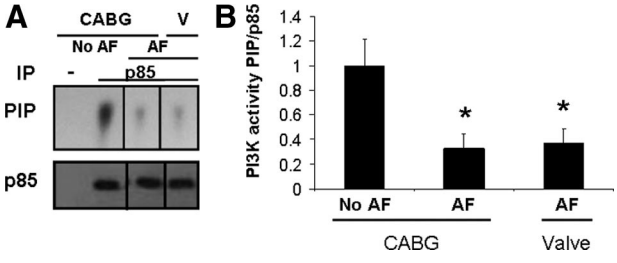

Figure 8. A: PI3K activity (**upper panel**) in atrial samples from patients undergoing CABG surgery who did or did not develop AF postoperatively (No AF) and a patient with mitral valve disease (V) and chronic AF. IP, immunoprecipitate; −, negative control (sample without antibody); PIP, phosphatidylinositol 3-phosphate. A portion of the immunoprecipitated antibody was subjected to Western blotting and probed with an anti-p85 antibody (**lower panel**). **B:** Quantitative analysis. NTg was normalized to 1.0. $^{*}P < 0.05$ versus No AF. CABG No AF: $N = 6$, CABG AF: $N = 4$, Valve AF: $N = 5$.

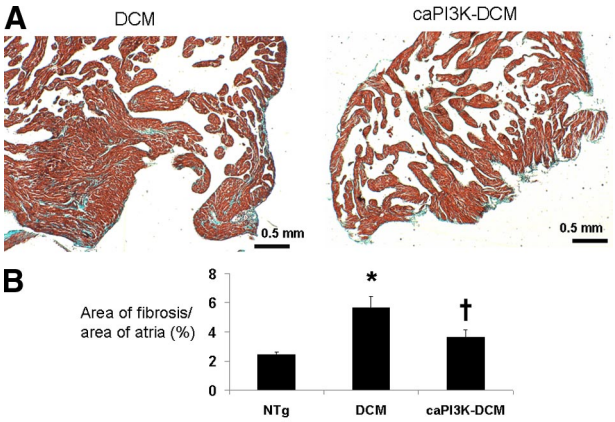

Figure 9. Atrial fibrosis in NTg, DCM-Tg, and caPI3K-DCM. **A:** Representative left atria showing fibrosis (blue staining Masson's trichrome). **B:** Quantitative analysis of area of fibrosis relative to area of the atria. $^{*}P < 0.05$ versus NTg; $^{\dagger}P < 0.05$ versus DCM. NTg: $N = 8$, DCM: $N = 7$, caPI3K-DCM: $N = 4$.

Enhanced PI3K(p110α) Activity Reverses Some of the Phenotypic Effects in DCM Mice

Data from both our mouse studies and human atrial appendages suggest that a reduction in PI3K(p110α) activity increases susceptibility toward AF. Next, we examined whether increasing PI3K(p110α) activity could reverse some of the features associated with the DCM phenotype. As DCM (4.5 months or aged mice ~15 months) do not develop AF we were unable to assess the incidence of AF directly. However, to address this question (at least in part) we assessed atrial fibrosis and the P-R interval in DCM mice with increased cardiac PI3K(p110α) activity. Both an increase in atrial fibrosis and increased P-R intervals (AV block) are associated with increased susceptibility to AF. PI3K activity was increased in hearts of DCM mice by breeding DCM mice with cardiac-specific transgenic mice with a caPI3K(p110α) mutant. caPI3K-DCM mice had reduced atrial fibrosis compared with DCM (Figure 9, A and B; 4.5 months) and reduced P-R intervals compared with DCM (caPI3K-DCM: 45 ± 2 versus DCM: 53 ± 2 ms; $P < 0.05$; $N = 5$ in each group; measurements at approximately 6.8 months in mice with similar heart rates caPI3K-DCM: 504 ± 15 bpm, DCM: 498 ± 9 bpm).

Discussion

Heart failure is a risk factor for the development of AF but the molecular factors responsible for the onset of AF in some patients but not others remains unclear. Similarly, there are numerous reports of heart failure mouse models but only a relatively small proportion of these models develop AF.[5,6,8–13] A greater understanding of the molecular mechanisms associated with heart failure induced AF, as well as the generation of genetic models to test therapeutic interventions is clearly needed. In this study, we examined whether a reduction in cardiac PI3K(p110α) signaling would accelerate heart failure in a well characterized model of DCM and whether this would make the heart susceptible to AF. PI3K(p110α) is a signaling protein that promotes physiological growth in the heart in response to exercise and protects the heart against cardiac dysfunction.[18,19] Risk factors for AF, including aging, obesity, and diabetes have all been associated with insulin resistance that leads to depressed/defective PI3K signaling.[46–48] However, to date, there has been no link between PI3K(p110α) activity and AF. The dnPI3K mouse model represents a genetic model with defective insulin signaling (in the heart alone).[49] Here, we report that reducing PI3K(p110α) activity in hearts of mice with DCM accelerated the progression of heart failure and led to the spontaneous onset of AF. Furthermore, data from atrial samples from patients suggest that reduced PI3K(p110α) activity may be a contributing factor to AF in humans. To our knowledge, this is the first study to implicate PI3K(p110α) activity as an important factor that can influence the incidence of AF.

dnPI3K-DCM expressing both DCM and dnPI3K had a phenotype that was worse than DCM-Tg alone. This is consistent with previous studies in which loss of PI3K(p110α) accelerated the progression of heart failure in a setting of pressure overload.[18,19] The dnPI3K-DCM model was associated with marked DCM and atrial enlargement and fibrosis. By ECG, all dnPI3K-DCM showed signs of severe AVB and depressed ECG potentials. AF was observed in ~40% of dnPI3K-DCM when ECGs were recorded for 5- to 10-minute intervals, although telemetry studies suggest the incidence is much higher. There is clinical and experimental evidence for atrial enlargement and fibrosis as key histopathological substrates for AF.[50] Both features were observed in our model and would increase the likelihood of re-entry and possibly contribute to the observed AF and AVB. We previously reported that activation of the insulin-like growth factor 1-PI3K(p110α) pathway protected the heart against fibrosis in response to pressure overload, whereas a reduction in PI3K(p110α) signaling lead to increased fibrosis.[19,20] In the current study, a reduction in PI3K activity was associated with an increase in atrial fibrosis and up-regulation of collagen genes in atria of dnPI3K-DCM. Thus, the dnPI3K transgene, when introduced on a background of DCM, may increase the likelihood of AF by increasing fibrosis.

An arrhythmic phenotype was not reported in the initial characterization of the DCM-Tg, but ECG studies were not performed.[33] In our study, DCM-Tg (~4.5 months) displayed AVB and had depressed R- and P-amplitudes; however, these changes were less prominent than that observed in dnPI3K-DCM. AF was not detected in DCM-Tg. It could be argued that reducing expression of a cardioprotective kinase, eg, PI3K(p110α), simply accelerated heart failure progression to induce AF in dnPI3K-DCM. To help address this question we also studied aged DCM-Tg (~15 months) with a similar heart failure phenotype to dnPI3K-DCM. The R-amplitude was more dramatically reduced in aged DCM-Tg than younger DCM-Tg (~4.5 months), however this change was still less pronounced than that observed in dnPI3K-DCM, and AF was not detected in aged DCM-Tg. It is also noteworthy that other mouse models with DCM and heart failure do not neces-

sarily develop AF. For instance, cardiac overexpression of β_2-adrenergic receptors caused a similar DCM phenotype to dnPI3K-DCM (including severe myocardial fibrosis and atrial enlargement) but ventricular tachyarrhythmia rather than AF and AVB was observed.[34] Finally, the accelerated heart failure phenotype observed in dnPI3K-DCM was ultimately a consequence of expression of the dnPI3K transgene reducing PI3K activity. Together, with the significant impact of the dnPI3K transgene alone on the P-R interval of aged mice and gene expression of potassium channels and metabolism genes, this suggests that loss of PI3K activity significantly contributed to the AF phenotype. Modifications in the expression of potassium channels and genes involved with energy metabolism have been linked with AF in animal models and humans.[41–43] Both the dnPI3K transgene and DCM transgene had a significant impact on gene expression of potassium channels and metabolism genes; these changes were even greater in dnPI3K-DCM. A limitation of the current study is that we were unable to provide protein expression data to support these findings due to the small size of mouse atria. Of note, other studies using larger species have reported good correlations between protein and gene expression of the Kv4.3 potassium channel (encoded by Kcnd3; Figure 7A), and down-regulation of this channel was associated with AF in humans and dogs.[51–54] PI3K(p110α) has also been reported to differentially regulate metabolism genes in ventricular tissue.[55] Further studies are required to examine the functional consequence of these alterations in gene expression.

To examine whether lowered PI3K activity in humans was also associated with AF, we assessed PI3K activity in atrial appendages from patients with acute or chronic AF. A relatively high proportion (10 to 50%) of patients undergoing cardiothoracic surgery develop paroxysmal AF postoperatively.[56,57] In the current study atrial PI3K activity was lower from this group than in patients who remained in sinus rhythm after surgery. PI3K activity was also reduced in atrial samples from patients with chronic AF. This suggests an association between reduced PI3K activity and the development of clinical AF. One could speculate that CABG surgery may represent a trigger that induces AF in patients with reduced cardiac PI3K activity under basal conditions (possibly as a consequence of the aging process or inactivity). This is consistent with the phenotype of the dnPI3K-Tg model that has normal cardiac function under basal conditions but more rapidly develops heart failure in response to a cardiac stress.[18,19]

Finally, to determine whether an increase in PI3K activity could reverse features associated with the DCM model that make it susceptible to AF in a setting of reduced PI3K activity, we generated double transgenic mice expressing the DCM transgene and a transgene that increases PI3K activity (caPI3K). Increasing PI3K activity in hearts of DCM mice reduced atrial fibrosis and improved cardiac conduction (reduced P-R interval). Future studies will assess whether increasing PI3K activity in other mouse models with AF can reduce or prevent the incidence of AF.

This study raises concerns regarding the use of PI3K(p110α) or tyrosine kinase inhibitors as anticancer agents in patients with underlying cardiac conditions.[32] We previously demonstrated that a reduction in PI3K(p110α) activity accelerated heart failure in setting of DCM or hypertrophic cardiomyopathy.[19] Our current work now raises concerns regarding an increased susceptibility to AF with the use of PI3K(p110α) inhibitors. Of note, a report of AF was described in a recent trial using lapatinib, a novel tyrosine kinase inhibitor, that acts in part by inhibiting PI3K.[58]

In summary, we have developed a genetic mouse model of AF that is associated with heart failure and overt atrial remodeling, simulating the clinical situation. A reduction in PI3K(p110α) activity, a critical effector of insulin signaling, can help explain the link between risk factors such as aging, obesity, and diabetes with AF. The relatively long lifespan of this genetic mouse model will allow for studies delineating molecular mechanisms leading to AF, identifying triggers of spontaneous AF and testing of therapeutic interventions. Strategies/agents that can activate PI3K(p110α) specifically in the heart may represent a therapeutic approach for heart failure and AF.

References

1. Fuster V, Ryden LE, Asinger RW, Cannom DS, Crijns HJ, Frye RL, Halperin JL, Kay GN, Klein WW, Levy S, McNamara RL, Prystowsky EN, Wann LS, Wyse DG, Gibbons RJ, Antman EM, Alpert JS, Faxon DP, Gregoratos G, Hiratzka LF, Jacobs AK, Russell RO, Smith SC, Alonso-Garcia A, Blomstrom-Lundqvist C, De Backer G, Flather M, Hradec J, Oto A, Parkhomenko A, Silber S, Torbicki A: ACC/AHA/ESC guidelines for the management of patients with atrial fibrillation: executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and the European Society of Cardiology Committee for Practice Guidelines and Policy Conferences (Committee to Develop Guidelines for the Management of Patients With Atrial Fibrillation): developed in Collaboration With the North American Society of Pacing and Electrophysiology. J Am Coll Cardiol 2001, 38:1231–1266
2. 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
3. Thrall G, Lane D, Carroll D, Lip GY: Quality of life in patients with atrial fibrillation: a systematic review. Am J Med 2006, 119:448 e441–419
4. Lip GY, Tse HF: Management of atrial fibrillation. Lancet 2007, 370:604–618
5. Hagendorff A, Schumacher B, Kirchhoff S, Luderitz B, Willecke K: Conduction disturbances and increased atrial vulnerability in Connexin40-deficient mice analyzed by transesophageal stimulation. Circulation 1999, 99:1508–1515
6. Temple J, Frias P, Rottman J, Yang T, Wu Y, Verheijck EE, Zhang W, Siprachanh C, Kanki H, Atkinson JB, King P, Anderson ME, Kupershmidt S, Roden DM: Atrial fibrillation in KCNE1-null mice. Circ Res 2005, 97:62–69
7. Zhang X, Chen S, Yoo S, Chakrabarti S, Zhang T, Ke T, Oberti C, Yong SL, Fang F, Li L, de la Fuente R, Wang L, Chen Q, Wang QK: Mutation in nuclear pore component NUP155 leads to atrial fibrillation and early sudden cardiac death. Cell 2008, 135:1017–1027
8. Sah VP, Minamisawa S, Tam SP, Wu TH, Dorn GW, 2nd, Ross J, Jr., Chien KR, Brown JH: Cardiac-specific overexpression of RhoA results in sinus and atrioventricular nodal dysfunction and contractile failure. J Clin Invest 1999, 103:1627–1634
9. Kehat I, Heinrich R, Ben-Izhak O, Miyazaki H, Gutkind JS, Aronheim A: Inhibition of basic leucine zipper transcription is a major mediator of atrial dilatation. Cardiovasc Res 2006, 70:543–554
10. Xiao HD, Fuchs S, Campbell DJ, Lewis W, Dudley SC, Jr., Kasi VS,

Hoit BD, Keshelava G, Zhao H, Capecchi MR, Bernstein KE: Mice with cardiac-restricted angiotensin-converting enzyme (ACE) have atrial enlargement, cardiac arrhythmia, and sudden death. Am J Pathol 2004, 165:1019–1032
11. Saba S, Janczewski AM, Baker LC, Shusterman V, Gursoy EC, Feldman AM, Salama G, McTiernan CF, London B: Atrial contractile dysfunction, fibrosis, and arrhythmias in a mouse model of cardiomyopathy secondary to cardiac-specific overexpression of tumor necrosis factor-α. Am J Physiol Heart Circ Physiol 2005, 289:H1456–1467
12. Adam O, Frost G, Custodis F, Sussman MA, Schafers HJ, Bohm M, Laufs U: Role of Rac1 GTPase activation in atrial fibrillation. J Am Coll Cardiol 2007, 50:359–367
13. Ogata T, Ueyama T, Isodono K, Tagawa M, Takehara N, Kawashima T, Harada K, Takahashi T, Shioi T, Matsubara H, Oh H: MURC, a muscle-restricted coiled-coil protein that modulates the Rho/ROCK pathway, induces cardiac dysfunction and conduction disturbance. Mol Cell Biol 2008, 28:3424–3436
14. Heist EK, Ruskin JN: Atrial fibrillation and congestive heart failure: risk factors, mechanisms, and treatment. Prog Cardiovasc Dis 2006, 48:256–269
15. Nattel S: New ideas about atrial fibrillation 50 years on. Nature 2002, 415:219–226
16. Shioi T, Kang PM, Douglas PS, Hampe J, Yballe CM, Lawitts J, Cantley LC, Izumo S: The conserved phosphoinositide 3-kinase pathway determines heart size in mice. EMBO J 2000, 19:2537–2548
17. McMullen JR, Jennings GL: Differences between pathological and physiological cardiac hypertrophy: novel therapeutic strategies to treat heart failure. Clin Exp Pharmacol Physiol 2007, 34:255–262
18. McMullen JR, Shioi T, Zhang L, Tarnavski O, Sherwood MC, Kang PM, Izumo S: Phosphoinositide 3-kinase(p110α) plays a critical role for the induction of physiological, but not pathological, cardiac hypertrophy. Proc Natl Acad Sci USA: 2003, 100:12355–12360
19. McMullen JR, Amirahmadi F, Woodcock EA, Schinke-Braun M, Bouwman RD, Hewitt KA, Mollica JP, Zhang L, Zhang Y, Shioi T, Buerger A, Izumo S, Jay PY, Jennings GL: Protective effects of exercise and phosphoinositide 3-kinase(p110α) signaling in dilated and hypertrophic cardiomyopathy. Proc Natl Acad Sci USA: 2007, 104:612–617
20. McMullen JR, Shioi T, Huang WY, Zhang L, Tarnavski O, Bisping E, Schinke M, Kong S, Sherwood MC, Brown J, Riggi L, Kang PM, Izumo S: The insulin-like growth factor 1 receptor induces physiological heart growth via the phosphoinositide 3-kinase(p110α) pathway. J Biol Chem 2004, 279:4782–4793
21. van der Hooft CS, Heeringa J, van Herpen G, Kors JA, Kingma JH, Stricker BH: Drug-induced atrial fibrillation. J Am Coll Cardiol 2004, 44:2117–2124
22. Kampinga HH, Henning RH, van Gelder IC, Brundel BJ: Beat shock proteins and atrial fibrillation. Cell Stress Chaperones 2007, 12:97–100
23. Mandal K, Torsney E, Poloniecki J, Camm AJ, Xu Q, Jahangiri M: Association of high intracellular, but not serum, heat shock protein 70 with postoperative atrial fibrillation. Ann Thorac Surg 2005, 79:865–871
24. St Rammos K, Koullias GJ, Hassan MO, Argyrakis NP, Voucharas CG, Scarupa SJ, Cowte TG: Low preoperative HSP70 atrial myocardial levels correlate significantly with high incidence of postoperative atrial fibrillation after cardiac surgery. Cardiovasc Surg 2002, 10:228–232
25. Afzal AR, Mandal K, Nyamweya S, Foteinos G, Poloniecki J, Camm AJ, Jahangiri M, Xu Q: Association of Met439Thr substitution in heat shock protein 70 gene with postoperative atrial fibrillation and serum HSP70 protein levels. Cardiology 2008, 110:45–52
26. Benjamin EJ, Levy D, Vaziri SM, D'Agostino RB, Belanger AJ, Wolf PA: Independent risk factors for atrial fibrillation in a population-based cohort. The Framingham Heart Study. JAMA 1994, 271:840–844
27. Wang TJ, Parise H, Levy D, D'Agostino RB, Sr., Wolf PA, Vasan RS, Benjamin EJ: Obesity and the risk of new-onset atrial fibrillation. JAMA 2004, 292:2471–2477
28. Lip GY, Varughese GI: Diabetes mellitus and atrial fibrillation: perspectives on epidemiological and pathophysiological links. Int J Cardiol 2005, 105:319–321
29. Perrino C, Prasad SV, Mao L, Noma T, Yan Z, Kim HS, Smithies O, Rockman HA: Intermittent pressure overload triggers hypertrophy-independent cardiac dysfunction and vascular rarefaction. J Clin Invest 2006, 116:1547–1560
30. Fan QW, Knight ZA, Goldenberg DD, Yu W, Mostov KE, Stokoe D, Shokat KM, Weiss WA: A dual PI3 kinase/mTOR inhibitor reveals emergent efficacy in glioma. Cancer Cell 2006, 9:341–349
31. Foukas LC, Claret M, Pearce W, Okkenhaug K, Meek S, Peskett E, Sancho S, Smith AJ, Withers DJ, Vanhaesebroeck B: Critical role for the p110alpha phosphoinositide-3-OH kinase in growth and metabolic regulation. Nature 2006, 441:366–370
32. McMullen JR, Jay PY: PI3K(p110alpha) inhibitors as anti-cancer agents: minding the heart. Cell Cycle 2007, 6:910–913
33. Yamamoto S, Yang G, Zablocki D, Liu J, Hong C, Kim SJ, Soler S, Odashima M, Thaisz J, Yehia G, Molina CA, Yatani A, Vatner DE, Vatner SF, Sadoshima J: Activation of Mst1 causes dilated cardiomyopathy by stimulating apoptosis without compensatory ventricular myocyte hypertrophy. J Clin Invest 2003, 111:1463–1474
34. Du XJ, Gao XM, Wang B, Jennings GL, Woodcock EA, Dart AM: Age-dependent cardiomyopathy and heart failure phenotype in mice overexpressing beta(2)-adrenergic receptors in the heart. Cardiovasc Res 2000, 48:448–454
35. Du XJ, Autelitano DJ, Dilley RJ, Wang B, Dart AM, Woodcock EA: beta(2)-adrenergic receptor overexpression exacerbates development of heart failure after aortic stenosis. Circulation 2000, 101:71–77
36. Head GA, Lukoshkova EV, Mayorov DN, van den Buuse M: Non-symmetrical double-logistic analysis of 24-h blood pressure recordings in normotensive and hypertensive rats. J Hypertens 2004, 22:2075–2085
37. Granelli-Piperno A, Reich E: A study of proteases and protease-inhibitor complexes in biological fluids. J Exp Med 1978, 148: 223–234
38. Liberatore GT, Samson A, Bladin C, Schleuning WD, Medcalf RL: Vampire bat salivary plasminogen activator (desmoteplase): a unique fibrinolytic enzyme that does not promote neurodegeneration. Stroke 2003, 34:537–543
39. Lekgabe ED, Kiriazis H, Zhao C, Xu Q, Moore XL, Su Y, Bathgate RA, Du XJ, Samuel CS: Relaxin reverses cardiac and renal fibrosis in spontaneously hypertensive rats. Hypertension 2005, 46:412–418
40. McMullen JR, Sherwood MC, Tarnavski O, Zhang L, Dorfman AL, Shioi T, Izumo S: Inhibition of mTOR signaling with rapamycin regresses established cardiac hypertrophy induced by pressure overload. Circulation 2004, 109:3050–3055
41. Ausma J, Coumans WA, Duimel H, Van der Vusse GJ, Allessie MA, Borgers M: Atrial high energy phosphate content and mitochondrial enzyme activity during chronic atrial fibrillation. Cardiovasc Res 2000, 47:788–796
42. 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
43. Tsai CT, Lai LP, Hwang JJ, Lin JL, Chiang FT: Molecular genetics of atrial fibrillation. J Am Coll Cardiol 2008, 52:241–250
44. Boixel C, Fontaine V, Rucker-Martin C, Milliez P, Louedec L, Michel JB, Jacob MP, Hatem SN: Fibrosis of the left atria during progression of heart failure is associated with increased matrix metalloproteinases in the rat. J Am Coll Cardiol 2003, 42:336–344
45. Spinale FG: Myocardial matrix remodeling and the matrix metalloproteinases: influence on cardiac form and function. Physiol Rev 2007, 87:1285–1342
46. Fink RI, Kolterman OG, Griffin J, Olefsky JM: Mechanisms of insulin resistance in aging. J Clin Invest 1983, 71:1523–1535
47. Tsang A, Hausenloy DJ, Mocanu MM, Carr RD, Yellon DM: Preconditioning the diabetic heart: the importance of Akt phosphorylation. Diabetes 2005, 54:2360–2364
48. Kahn BB, Flier JS: Obesity and insulin resistance. J Clin Invest 2000, 106:473–481
49. Hsueh W, Abel ED, Breslow JL, Maeda N, Davis RC, Fisher EA, Dansky H, McClain DA, McIndoe R, Wassef MK, Rabadan-Diehl C, Goldberg IJ: Recipes for creating animal models of diabetic cardiovascular disease. Circ Res 2007, 100:1415–1427
50. Roberts R: Genomics and cardiac arrhythmias. J Am Coll Cardiol 2006, 47:9–21
51. Borlak J, Thum T: Hallmarks of ion channel gene expression in end-stage heart failure. FASEB J 2003, 17:1592–1608
52. Brundel BJ, Van Gelder IC, Henning RH, Tuinenburg AE, Wietses M, Grandjean JG, Wilde AA, Van Gilst WH, Crijns HJ: Alterations in potassium channel gene expression in atria of patients with persistent and paroxysmal atrial fibrillation: differential regulation of protein and mRNA levels for K+ channels. J Am Coll Cardiol 2001, 37:926–932

53. Yue L, Melnyk P, Gaspo R, Wang Z, Nattel S: Molecular mechanisms underlying ionic remodeling in a dog model of atrial fibrillation. Circ Res 1999, 84:776–784
54. Grammer JB, Bosch RF, Kuhlkamp V, Seipel L: Molecular remodeling of Kv4.3 potassium channels in human atrial fibrillation. J Cardiovasc Electrophysiol 2000, 11:626–633
55. O'Neill BT, Kim J, Wende AR, Theobald HA, Tuinei J, Buchanan J, Guo A, Zaha VG, Davis DK, Schell JC, Boudina S, Wayment B, Litwin SE, Shioi T, Izumo S, Birnbaum MJ, Abel ED: A conserved role for phosphatidylinositol 3-kinase but not Akt signaling in mitochondrial adaptations that accompany physiological cardiac hypertrophy. Cell Metab 2007, 6:294–306
56. Chandy J, Nakai T, Lee RJ, Bellows WH, Dzankic S, Leung JM: Increases in P-wave dispersion predict postoperative atrial fibrillation after coronary artery bypass graft surgery. Anesth Analg 2004, 98:303–310
57. Aranki SF, Shaw DP, Adams DH, Rizzo RJ, Couper GS, VanderVliet M, Collins JJ, Jr., Cohn LH, Burstin HR: Predictors of atrial fibrillation after coronary artery surgery: current trends and impact on hospital resources. Circulation 1996, 94:390–397
58. Blackwell KL, Pegram MD, Tan-Chiu E, Schwartzberg LS, Arbushites MC, Maltzman JD, Forster JK, Rubin SD, Stein SH, Burstein HJ: Single-agent lapatinib for HER2-overexpressing advanced or metastatic breast cancer that progressed on first- or second-line trastuzumab-containing regimens. Ann Oncol 2009, 20:1026–1031