Upregulation of $K_{2P}3.1$ K^+ Current Causes Action Potential Shortening in Patients With Chronic Atrial Fibrillation

Constanze Schmidt, MD; Felix Wiedmann, MD; Niels Voigt, MD; Xiao-Bo Zhou, MD; Jordi Heijman, PhD; Siegfried Lang, PhD; Virginia Albert, BSc; Stefan Kallenberger, MD, PhD; Arjang Ruhparwar, MD; Gábor Szabó, MD, PhD; Klaus Kallenbach, MD; Matthias Karck, MD; Martin Borggrefe, MD; Peter Biliczki, MD, PhD; Joachim R. Ehrlich, MD; István Baczkó, MD, PhD; Patrick Lugenbiel, MD; Patrick A. Schweizer, MD; Birgit C. Donner, MD, PhD; Hugo A. Katus, MD, PhD; Dobromir Dobrev, MD*; Dierk Thomas, MD*

Background—Antiarrhythmic management of atrial fibrillation (AF) remains a major clinical challenge. Mechanism-based approaches to AF therapy are sought to increase effectiveness and to provide individualized patient care. $K_{2P}3.1$ (TASK-1 [tandem of P domains in a weak inward-rectifying K^+ channel–related acid-sensitive K^+ channel-1]) 2-pore-domain K^+ (K_{2P}) channels have been implicated in action potential regulation in animal models. However, their role in the pathophysiology and treatment of paroxysmal and chronic patients with AF is unknown.

Methods and Results—Right and left atrial tissue was obtained from patients with paroxysmal or chronic AF and from control subjects in sinus rhythm. Ion channel expression was analyzed by quantitative real-time polymerase chain reaction and Western blot. Membrane currents and action potentials were recorded using voltage- and current-clamp techniques. $K_{2P}3.1$ subunits exhibited predominantly atrial expression, and atrial $K_{2P}3.1$ transcript levels were highest among functional K_{2P} channels. $K_{2P}3.1$ mRNA and protein levels were increased in chronic AF. Enhancement of corresponding currents in the right atrium resulted in shortened action potential duration at 90% of repolarization (APD_{90}) compared with patients in sinus rhythm. In contrast, $K_{2P}3.1$ expression was not significantly affected in subjects with paroxysmal AF. Pharmacological $K_{2P}3.1$ inhibition prolonged APD_{90} in atrial myocytes from patients with chronic AF to values observed among control subjects in sinus rhythm.

Conclusions—Enhancement of atrium-selective $K_{2P}3.1$ currents contributes to APD shortening in patients with chronic AF, and $K_{2P}3.1$ channel inhibition reverses AF-related APD shortening. These results highlight the potential of $K_{2P}3.1$ as a novel drug target for mechanism-based AF therapy. **(*Circulation*. 2015;132:82-92. DOI: 10.1161/CIRCULATIONAHA.114.012657.)**

Key Words: arrhythmias, cardiac ■ atrial fibrillation ■ electrophysiology

Successful, safe pharmacological treatment of atrial fibrillation (AF) is a primary yet unmet need in cardiovascular medicine.[1] Patients with AF exhibit largely variable disease characteristics and continue to be at high risk for hospitalizations, heart failure, and stroke as a result of the limited effectiveness of unspecific pharmacological or interventional treatment. Patient-tailored therapy is required to improve the outcomes of patients with AF. However, mechanism-based approaches are currently limited by an insufficient understanding of precise molecular remodeling associated with AF. Shortening of action potential (AP) duration (APD) is considered a hallmark of atrial remodeling in AF that promotes re-entry, supporting the perpetuation of the arrhythmia.[2] The therapeutic significance of accelerated atrial repolarization is highlighted by AF suppression through

Clinical Perspective on p 92

Received August 5, 2014; accepted May 1, 2015.

From Department of Cardiology, University of Heidelberg, Germany (C.S., F.W., V.A., P.L., P.A.S., H.A.K., D.T.); Division of Experimental Cardiology, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany (N.V., X.-B.Z., J.H., S.L., M.B., D.D.); Institute of Pharmacology, Faculty of Medicine, University Duisburg-Essen, Essen, Germany (N.V., J.H., D.D.); First Department of Medicine, University Medical Center Mannheim, Germany (X.-B.Z., S.L., M.B.); Department for Bioinformatics and Functional Genomics, Division of Theoretical Bioinformatics, German Cancer Research Center, Institute for Pharmacy and Molecular Biotechnology and BioQuant, Heidelberg University, Germany (S.K.); Department of Cardiac Surgery, University Hospital Heidelberg, Germany (A.R., G.S., K.K., M.K.); Department of Cardiology, Internal Medicine III, Goethe University, Frankfurt, Germany (P.B., J.R.E.); Division of Cardiology, Deutsche Klinik für Diagnostik, Wiesbaden, Germany (P.B., J.R.E.); Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Szeged, Hungary (I.B.); and Department of Cardiology, University of Basel Children's Hospital, Switzerland (B.C.D.).

*Drs Dobrev and Thomas contributed equally.

The online-only Data Supplement is available with this article at http://circ.ahajournals.org/lookup/suppl/doi:10.1161/CIRCULATIONAHA.114.012657/-/DC1.

Correspondence to Dierk Thomas, MD, FAHA, FESC, FHRS, Department of Cardiology, University of Heidelberg, Im Neuenheimer Feld 410, D-69120 Heidelberg, Germany. E-mail dierk.thomas@med.uni-heidelberg.de

Circulation **is available at http://circ.ahajournals.org** **DOI: 10.1161/CIRCULATIONAHA.114.012657**

inhibition of repolarizing K^+ currents by class III antiarrhythmic drugs or via targeted gene transfer.[3,4] Although constitutive $I_{K,ACh}$ activity, increased I_{K1} current, and decreased $I_{Ca,L}$ have previously been implicated in APD shortening during AF, the contribution of other ion channels is poorly understood.[2,5–8]

Two-pore-domain K^+ (K_{2P}) channels facilitate AP repolarization, and regulation of K_{2P} currents dynamically determines cellular excitability.[9–16] Specifically, cardiac $K_{2P}3.1$ (TASK-1 [tandem of P domains in a weak inward-rectifying K^+ channel–related acid-sensitive K^+ channel-1]) currents are implicated in AP regulation and may contribute to AF.[17–22] Inhibition or genetic inactivation of cardiac $K_{2P}3.1$ channels results in APD prolongation in rodents.[17–20] In the human heart, $K_{2P}3.1$ K^+ channels are expressed predominantly in the atria and could serve as atrium-specific antiarrhythmic targets for AF therapy.[23,24] A role for cardiac $K_{2P}3.1$ channels as drug targets is further supported by their sensitivity to established antiarrhythmic compounds.[25–29] The aim of this study was to explore the potential contribution of $K_{2P}3.1$ current dysregulation to AF-related APD abbreviation and to assess the relevance of $K_{2P}3.1$ inhibition for mechanism-based therapy in patients with paroxysmal AF (pAF) and chronic AF (cAF).

Methods

Study Patients

A total of 122 patients (mean age, 68±12 years; male/female, 83/39) with sinus rhythm (SR; n=39), pAF (n=39), and cAF (ie, persistent, long-standing persistent, or permanent AF; n=44) undergoing open heart surgery for coronary artery bypass grafting or valve repair/replacement were included (Table). Tissue samples were obtained from the right or left atrial appendage. For comparison, left ventricular (LV) tissue samples were acquired from 5 patients with ischemic or dilated cardiomyopathy during LV assist device implantation to evaluate ventricular expression levels. All patients received sevoflurane for general anesthesia. The study protocol involving human tissue samples was approved by the ethics committees of the University of Heidelberg (Germany; Medical Faculty Heidelberg, S-017/2013; Medical Faculty Mannheim, 2011-216 N-MA), the University of Frankfurt am Main (Germany; 53/08), and the University of Szeged (Hungary; license number 717, reference number 63/97). Written informed consent was obtained from all patients, and the study was conducted in accordance with the Declaration of Helsinki.

Quantitative Real-Time Polymerase Chain Reaction

Quantitative real-time polymerase chain reaction was performed with the StepOnePlus (Applied Biosystems, Foster City, CA) polymerase chain reaction system according to the manufacturer's protocol. All quantitative real-time polymerase chain reactions were performed in triplicate (see Table I in the online-only Data Supplement for primer information), and control experiments in the absence of cDNA were included. Data are expressed as an average of triplicates.

Western Blot Analysis

Protein immunodetection was performed by SDS gel electrophoresis and Western blotting with primary antibodies directed against $K_{2P}3.1$ (1:200; APC-024; Alomone Labs, Jerusalem, Israel), as described.[30–33] Protein content was normalized to GAPDH.

Isolation of Atrial Myocytes

Myocytes were enzymatically dispersed with collagenase essentially as reported (see Supplemental Methods in the online-only Data Supplement for details).[34,35]

Cellular Electrophysiology

Current and membrane potential recordings from cardiac myocytes were carried out at room temperature (21°C–25°C) with an RK-400 amplifier (Bio-Logic SAS) using the whole-cell patch clamp configuration as published.[15] The $K_{2P}3.1$ channel inhibitor A293 {2-(butane-1-sulfonylamino)-N-[1-(R)-(6-methoxypyridin-3-yl)-propyl]-benzamide}[17] (kindly provided by Sanofi-Aventis, Berlin, Germany) was applied to isolate $K_{2P}3.1$ current. A293 was dissolved in dimethyl sulfoxide to a stock solution of 10 mmol/L and stored at −20°C. Cardiac APs were recorded from freshly isolated myocytes using the whole-cell patch-clamp technique at room temperature (21°C–25°C). APs were elicited in current-clamp mode with a holding current of −40 pA by injection of brief current pulses (2 milliseconds, 1 nA) at a 0.2-Hz stimulation rate.

Computational Modeling

The SR and cAF versions of the Grandi et al[36] computational model of the human atrial cardiomyocyte, including our recent update with Na^+-dependent regulation of I_{K1} and $I_{K,ACh}$,[37] was extended with a formulation for the $K_{2P}3.1$ current (Supplemental Methods, Table II, and Figure I in the online-only Data Supplement).

Data Acquisition and Statistical Analysis

Data acquisition was performed with pClamp software (Molecular Devices, Sunnyvale, CA). Origin 6 (OriginLab, Northampton, MA) software was used for data analysis. Patient data are expressed as mean±SD. Data obtained from patch-clamp recordings are provided as mean±SEM. Statistical significance between means of continuous variables was evaluated with the Student *t* tests. Values of $P<0.05$ were considered statistically significant. Multiple comparisons were performed with 1-way ANOVA. The Bonferroni adjustment was used for post hoc testing. If a quantity was dependent on 2 attributes (ie, to analyze correlations between channel expression and rhythm or LV function), we performed a 2-factor ANOVA to assess the main effects of the factors and their interaction. Similarly, 2-factor repeated-measures ANOVA was applied when multiple measurements were taken on individual myocytes at different membrane voltages. To test for rank-order correlation, we calculated the Kendall τ.

Results

K_{2P} Channel Expression in the Human Heart

A comprehensive expression analysis of all human K_{2P} isoforms identified $K_{2P}1.1$ and $K_{2P}3.1$ as predominant K_{2P} subunits in the right and left atria of patients with SR (n=14; Figure 1). $K_{2P}3.1$ channels were studied in detail in the present study owing to robust atrial expression in combination with pronounced AF-associated remodeling that was unique among K_{2P} channels (Figure 1). In LV tissue samples (n=5), $K_{2P}3.1$ transcript levels were low compared with the right atrium (16-fold; n=5–10; $P<0.0001$) and left atrium (14-fold; n=4; $P=0.066$; Figure 1). For comparison, ion channel genes with established significance in human atrial electrophysiology and arrhythmogenesis were analyzed, revealing that atrial $K_{2P}3.1$ mRNA expression was similar to $K_v4.3$ channels conducting the cardiac transient outward K^+ current and to inward-rectifier potassium channels $K_{ir}2.2$ and $K_{ir}2.3$ (Figure 2).

Increased $K_{2P}3.1$ Levels Contribute to Atrial Remodeling in Patients With cAF

Remodeling of ion channel expression is generally believed to constitute the electric substrate that shortens atrial APD, supporting AF-maintaining re-entry. We found that $K_{2P}3.1$ mRNA expression in the right atrium was elevated by 59.8%

Table. Baseline Characteristics of Study Patients

	RAA			LAA		
	SR (n=35)	pAF (n=33)	cAF (n=33)	SR (n=4)	pAF (n=6)	cAF (n=11)
Demographics						
Men, n (%)	25 (71)	19 (76)	26 (79)	3 (75)	4 (67)	6 (55)
Age, y	63.7±13.8	71.7±12.6*	70.3±7.9	63.0±2.9	64.8±11.6	70.3±5.7
Body mass index, kg/m^2	28.1±4.8	27.9±4.6	27.9±5.3	NA	NA	NA
Height, cm	170±9.6	171±10.7	173±7.8	NA	NA	NA
Medical history, n (%)						
CAD	24 (69)	21 (64)	19 (58)	0 (0)	0 (0)†	0 (0)†
AVD	15 (43)	16 (49)	24 (73)*	0 (0)	0 (0)	0 (0)†
MVD	0 (0)	0 (0)	0 (0)	4 (100)	6 (100)†	11 (100) †
CAD+AVD	4 (11)	4 (12)	10 (30)	0 (0)	0 (0)	0 (0)
Hypertension	34 (97)	28 (85)	31 (94)	3 (75)	0 (0) *†	4 (36)†
Diabetes mellitus	12 (34)	7 (21)	10 (30)	1 (25)	0 (0)	3 (27)
Hyperlipidemia	25 (71)	21 (64)	27 (82)	1 (25)	1 (17)	6 (55)
LVEF, n (%)						
Normal	21 (60)	15 (45)	10 (30)*	2 (50)	4 (67)	7 (64)
Mild reduced	6 (17)	7 (21)	7 (21)	0 (0)	0 (0)	4 (36)
Moderate reduced	5 (14)	6 (18)	10 (30)	1 (25)	2 (33)	0 (0)
Severe reduced	3 (9)	5 (15)	6 (18)	1 (25)	0 (0)	0 (0)
Concomitant medication, n (%)						
Digitalis	1 (3)	3 (9)	7 (21) *	0 (0)	1 (17)	2 (18)
ACE inhibitors	22 (63)	16 (49)	14 (42)	4 (100)	3 (50)	9 (82)†
AT1 antagonists	6 (17)	5 (15)	7 (21)	NA	NA	NA
β-Blockers	24 (69)	25 (76)	24 (73)	3 (75)	5 (83)	6 (55)
Diuretics	13 (37)	20 (61)	28 (85) *	NA	NA	NA
Nitrates	0 (0)	3 (9)	2 (6)	NA	NA	NA
Lipid-lowering drugs	22 (63)	24 (73)	20 (61)	2 (50)	1 (17)†	5 (46)
OAC	9 (26)	22 (67) *	23 (70) *	2 (50)	5 (83)	9 (82)

ACE indicates angiotensin-converting enzyme; AT1, angiotensin receptor-1; AVD, aortic valve disease; CAD, coronary artery disease; cAF, chronic atrial fibrillation; LAA, left atrial appendage; LVEF, left ventricular ejection fraction (normal, ≥55%; mild impairment, 45%–54%; moderate impairment, 30%–44%; severe impairment, <30%); MVD, mitral valve disease; NA, not available; OAC, oral anticoagulation; pAF, paroxysmal atrial fibrillation; RAA, right atrial appendage; and SR, sinus rhythm.

*P<0.05 vs SR, †P<0.05 versus corresponding values in the RA from ANOVA followed by Bonferroni multiple-comparisons procedure for continuous variables and from the Fisher exact test for categorical variables.

(P=0.030) in patients with cAF (n=10) compared with individuals with SR (n=10; Figure 1). In addition, there was a 27.6% increase of $K_{2P}3.1$ mRNA levels in left atrial tissue (cAF, n=11 versus SR, n=4) that was not statistically significant (P=0.55; Figure 1). In contrast, $K_{2P}3.1$ mRNA levels did not change in patients with pAF (n=16) compared with patients in SR (n=14; Figure 1). Alterations of $K_{2P}3.1$ mRNA expression levels were consistent with $K_{2P}3.1$ immunoblots (Figure 3 and Figure II in the online-only Data Supplement). cAF was associated with upregulation of $K_{2P}3.1$ immunoreactivity at 50 to 55 kDa, corresponding to the fully processed membrane protein, in the right atrium by 64.0±17.7% (P=0.025; n=4) compared with patients in SR (Figure 3A–3C). We also observed a moderate increase in $K_{2P}3.1$ protein expression in pAF (37.4±13.1%; P=0.043; n=4). Of note, $K_{2P}3.1$ immunosignal intensity at ≈200 kDa, which may reflect channel aggregates, was similarly upregulated in patients with cAF (Figure 3A). Low protein levels were detected by anti-$K_{2P}3.1$ antibodies in an exemplary ventricular sample, highlighting weak $K_{2P}3.1$ expression in LV tissue (Figure IIIA in the online-only Data Supplement). However, limited discrimination of $K_{2P}3.1$ and other cardiac proteins by anti-$K_{2P}3.1$ antibodies in mice requires cautious attention in the interpretation of human Western blot data (the online-only Data Supplement provides an in-depth appraisal of antibody specificity).

In addition to $K_{2P}3.1$, K_{2P} channels $K_{2P}13.1$ and $K_{2P}17.1$ were significantly affected in patients with cAF, displaying reduced mRNA levels in the right atrium (Figure 1). cAF was further associated with significant upregulation ($K_{ir}2.1$; KCNQ1) or suppression (sulfonylurea receptor 1, potassium channel-interacting protein 2, $K_{ir}3.1$, $K_{ir}3.4$) of additional ion

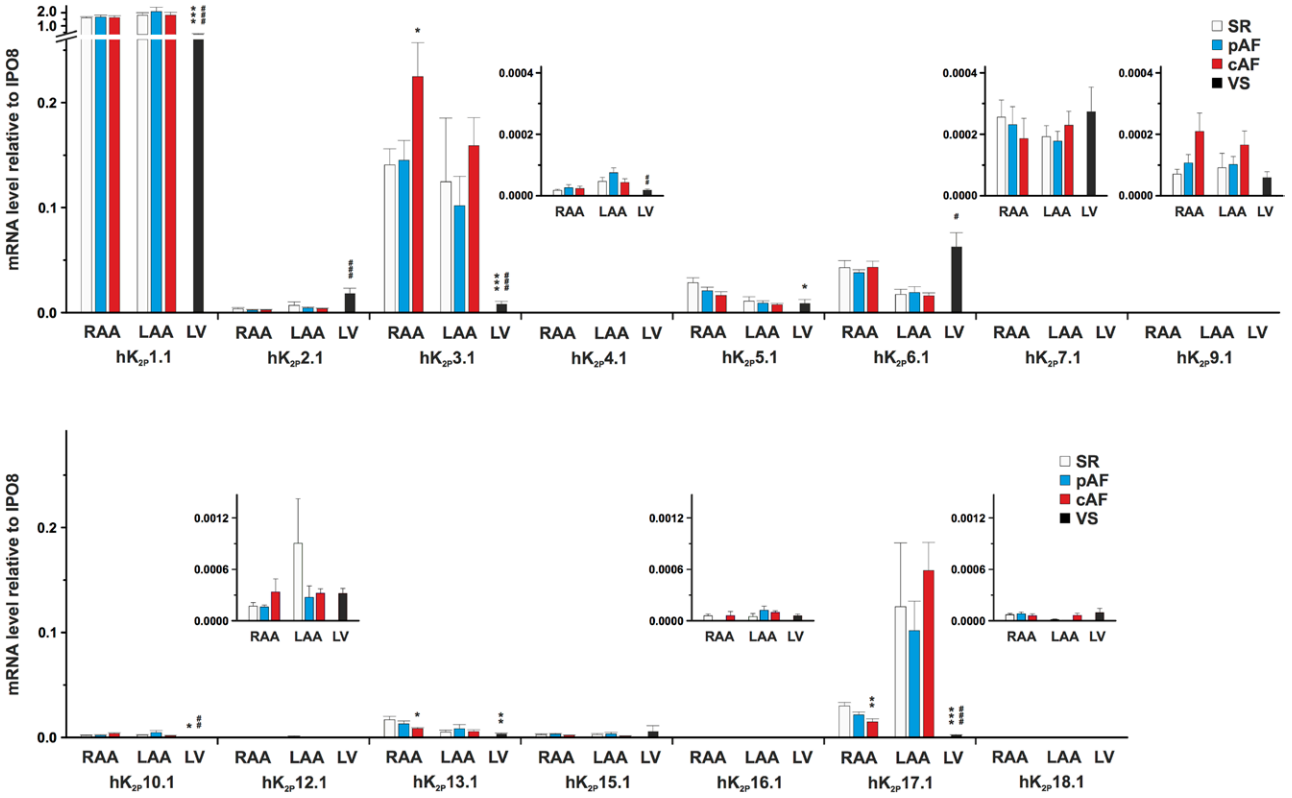

Figure 1. Cardiac expression and remodeling of K_{2P} channel mRNA in patients with sinus rhythm (SR), paroxysmal atrial fibrillation (pAF), and chronic atrial fibrillation (cAF). Ventricular samples (VS) were analyzed for comparison. Insets represent selective enlargements to visualize transcript levels of subunits with low expression. Note that the function of $K_{2P}1.1$, $K_{2P}7.1$, $K_{2P}12.1$, and $K_{2P}15.1$ protein has not been unequivocally established to date. Data are expressed as mean±SEM arbitrary units normalized to IPO8. IPO8 indicates importin 8; LAA, left atrial appendage; LV, left ventricle; and RAA, right atrial appendage. **P*<0.05, ***P*<0.01, ****P*<0.001 vs RAA/SR; #*P*<0.05, ##*P*<0.01, ###*P*<0.001 vs LAA/SR.

channels and accessory subunits relevant to atrial electrophysiology (Figure 2). Of note, we did not detect significant electric remodeling in patients with pAF.

$K_{2P}3.1$ Current Enhancement in cAF

Functional consequences of $K_{2P}3.1$ upregulation were studied in right atrial myocytes obtained from patients with SR, pAF, and cAF. $K_{2P}3.1$ current was isolated by use of the experimental compound A293, which specifically inhibits the channels at 200 nmol/L (Figure 4A; see also Supplemental Results and Figure IV in the online-only Data Supplement).[18,24] A293-sensitive K^+ currents activated at potentials >−20 mV and showed Goldman-Hodgkin-Katz (open or outward) rectification that is characteristic of K_{2P} channels (Figure 4B–4F). $K_{2P}3.1$ current density quantified at 40 mV was increased by 3.1-fold in patients with cAF (n=13 cells obtained from N=5 individuals) compared with SR (n/N=17/6; *P*=0.002; Figure 4F and 4G; see Figure V in the online-only Data Supplement for absolute current values and cell capacitance data). $K_{2P}3.1$ currents tended to be 1.5-fold higher in pAF subjects (n/N=13/6) in relation to SR (n/N=17/6) without statistical significance (*P*=0.47; Figure 4E and 4G).

$K_{2P}3.1$ Upregulation Is Associated With APD Shortening

Upregulation of $K_{2P}3.1$ mRNA, protein, and corresponding currents in cAF suggest functional relevance in shaping the atrial AP. Atrial APs were studied under current-clamp conditions in human atrial myocytes. APD at 90% of repolarization (APD_{90}) was abbreviated by 42.9% from 213.0±11.1 milliseconds (SR; n/N=9/6) to 121.7±12.6 milliseconds (cAF; n/N=10/6; *P*<0.0001; Figure 5A, 5C, and 5E) in cAF, consistent with the increase in repolarizing $K_{2P}3.1$ currents. In patients with pAF (n/N=9/5), APD_{90} remained virtually unchanged in relation to SR (*P*=0.67; Figure 5B, 5D, and 5E). There was no rhythm-dependent modulation of APD at 50% of repolarization (APD_{50}; Figure 5A–5D) or resting membrane potential (Figure V in the online-only Data Supplement) in any group.

Class III Antiarrhythmic Effects of $K_{2P}3.1$ Channel Inhibition in cAF Patients

The experimental $K_{2P}3.1$ inhibitor A293 was used to test the hypothesis that pharmacological $K_{2P}3.1$ reduction would reverse APD shortening in cAF. In human atrial myocytes obtained from patients in SR (n/N=9/6), $K_{2P}3.1$ block by 200 nmol/L A293 induced only a weak prolongation of APD_{50} (3.4±1.6%; *P*=0.11) and APD_{90} (17.1±4.5%; *P*=0.012; Figure 5A and 5D–5F). In contrast, APD_{90} was markedly prolonged by 57.9±10.0% (n/N=10/6) in cAF (200 nmol/L A293; *P*<0.0001), indicating significant class III antiarrhythmic efficacy in this subset of patients with AF (Figure 5C, 5E, and 5F). A293 also increased APD_{90} in pAF, albeit to a lesser degree (27.8±6.3%; *P*=0.003; Figure 5B, 5E, and 5F). A direct

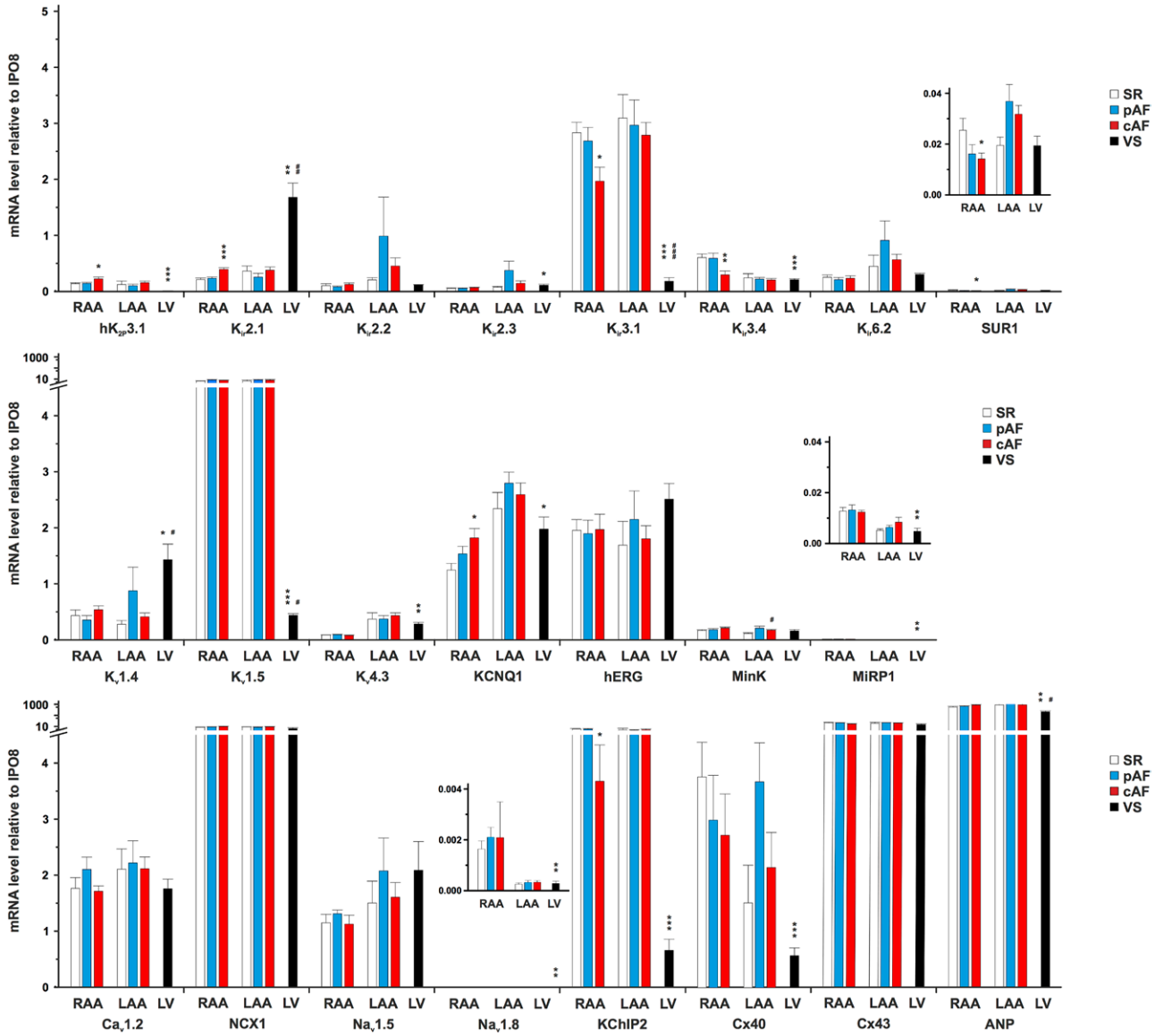

Figure 2. Atrial expression profile of indicated ion channel subunits in patients with sinus rhythm (SR), paroxysmal atrial fibrillation (pAF), and chronic atrial fibrillation (cAF). Data obtained from ventricular samples (VS) were analyzed for reference. Insets represent selective enlargements to visualize low-level transcripts. Data are expressed as mean±SEM arbitrary units normalized to IPO8. ANP indicates atrial natriuretic peptide; Cx40, connexin40; Cx43, connexin43; hERG, human ether-a-go-go–related gene; IPO8, importin 8; KChIP, potassium channel-interacting protein; LAA, left atrial appendage; LV, left ventricle; minK, minimal K^+ channel; MiRP, minK-related peptide; NCX, sodium-calcium exchanger; RAA, right atrial appendage; and SUR, sulfonylurea receptor. **P*<0.05, ***P*<0.01, ****P*<0.001 vs RAA/SR; #*P*<0.05, ##*P*<0.01, ###*P*<0.001 vs LAA/SR.

comparison of the A293 effects between patients with SR, pAF, and cAF revealed that specific $K_{2P}3.1$ blockade had little effect on absolute APD_{90} in SR and pAF (Figure 5E), whereas in patients with cAF, A293 increased APD_{90} to APD levels typical for SR subjects (Figure 5E and 5F).

Computational Analysis of the Effect of $K_{2P}3.1$ Current on APD in SR and cAF

The Grandi et al[36] computational model of the human atrial cardiomyocyte was extended with a formulation for the $K_{2P}3.1$ current based on the experimentally measured I-V relationship (Figure I in the online-only Data Supplement). The SR and cAF versions of the model were adjusted to reproduce the experimental APD_{50} and APD_{90} under simulated conditions corresponding to the experimentally used pipette and bath solutions (Figure 6A and 6B). Simulated inhibition of $K_{2P}3.1$ channels produced a modest prolongation of APD_{90} in the SR model but a much larger prolongation in the cAF model (Figure 6A and 6C), consistent with experimental results. Moreover, this APD prolongation was observed at all pacing frequencies between 0.2 and 3.3 Hz (Figure 6D). Finally, APD in the cAF model after $K_{2P}3.1$ channel blockade approached that of the SR model, with a reduction in the APD difference from 93.2 to 28.1 milliseconds (−70%) after $K_{2P}3.1$ channel blockade compared with SR simulations. Together, these data suggest that, under these conditions, upregulation of $K_{2P}3.1$ in patients with cAF plays a major role in the proarrhythmic APD shortening.

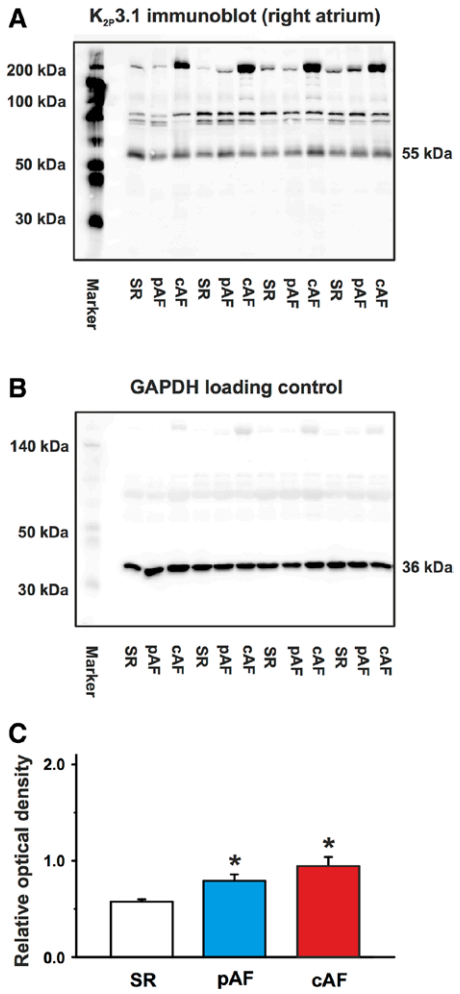

Figure 3. Western blot analysis of $K_{2P}3.1$ protein in human right atrium. **A**, Representative immunoblots obtained from patients in sinus rhythm (SR), paroxysmal atrial fibrillation (pAF), or chronic atrial fibrillation (cAF) probed with anti-$K_{2P}3.1$ antibodies. **B**, Anti-GAPDH antibodies were applied to quantify protein load. **C**, Mean±SEM optical density values normalized to GAPDH expression of indicated patient groups (n=4 subjects per group; **P*<0.05 vs SR).

Independent Effects of Cardiac Function on Atrial $K_{2P}3.1$ Expression

To provide a more precise characterization of the patient population likely to benefit from $K_{2P}3.1$ blockade, the correlation of right atrial $K_{2P}3.1$ expression levels with LV function was explored. Patient groups with SR (n=16), pAF (n=12), and cAF (n=11) were analyzed. Study subgroups were not significantly different with respect to sex, body mass index, or medical history. The potential relationship between $K_{2P}3.1$ levels and LV function or rhythm was statistically analyzed via 2-way ANOVA with rhythm status (SR, pAF, cAF) and LV function (normal; mild, moderate, severe reduction) as factors. The analysis revealed a significant association between LV function of study patients and $K_{2P}3.1$ expression (F=53.6; P=0.006; Figure 7A). Atrial $K_{2P}3.1$ levels were significantly downregulated in patients with severe LV function impairment regardless of the rhythm status compared with no, mild, and medium impairment (P=0.047; Figure 7A). This is in contrast to the correlation between rhythm status and $K_{2P}3.1$ expression (F=42.3; P=0.026) characterized by cAF-associated upregulation (P=0.022; Figure 7B). There was no significant correlation between LV function and cardiac rhythm (F=11.8; P=0.35; Kendall τ=−0.16) in the patient cohort.

Discussion

Atrial $K_{2P}3.1$ K^+ Channels in Humans With SR

K_{2P} potassium channels conduct repolarizing currents and contribute to the resting membrane voltage in excitable cells.[9] In the present work, we delineated mRNA expression of multiple K_{2P} channels in left and right atria obtained from control subjects with SR. $K_{2P}3.1$ displayed highest transcript levels among K_{2P} family members with confirmed K^+ channel function (ie, after exclusion of $K_{2P}1.1$, $K_{2P}7.1$, $K_{2P}12.1$, and $K_{2P}15.1$ subunits, which do not produce substantial K^+ currents) and was specifically studied. The high ratio of atrial to ventricular $K_{2P}3.1$ transcripts (16:1) highlighted predominantly atrial expression. Inhibition of $K_{2P}3.1$ current produced a tendency toward prolonged APD_{90} by 17% in patients in SR, reflecting class III antiarrhythmic effects. These data indicate that $K_{2P}3.1$ functionally contributes to the atrial AP in subjects with SR and represents an atrium-selective target for antiarrhythmic therapy.

APD Shortening in cAF Patients: Significance of $K_{2P}3.1$ and Comparison With Previous Studies

Electric remodeling of human atrial tissue is a hallmark of AF pathophysiology, stabilizing re-entrant circuits via abbreviation of atrial APD.[2] We observed significant shortening of APD_{90} in patients with cAF compared with subjects with SR. In contrast, there was no APD reduction in pAF cardiomyocytes, in accordance with previous data.[38] In addition, the patients' rhythm status was not associated with atrial resting membrane potential changes in the present study consistent with earlier work.[35,38,39] Similarly, inhibition of $K_{2P}3.1$ current had no effect on resting membrane potential. The molecular basis of electric remodeling was further elucidated in a comprehensive approach that included all K_{2P} channels and 21 additional ion channel subunits relevant to atrial electrophysiology. The main finding was a significant upregulation of $K_{2P}3.1$ expression and current levels in patients with cAF but not in patients with pAF, suggesting a mechanistic explanation for the typical APD shortening in patients with cAF. The presence of noninactivating outward K^+ currents in patient-derived atrial myocytes after extensive pharmacological block of established potassium channels additionally highlights a significant contribution of $K_{2P}3.1$ conductance to human cardiac electrophysiology.[40]

AF-associated APD shortening has previously been attributed to increased I_{K1} current, downregulation of $I_{Ca,L}$, and constitutively active $I_{K,ACh}$ (despite decreased $K_{ir}3.1$ and $K_{ir}3.4$ subunits underlying the current).[2,41,42] In the present cAF cohort, APD abbreviation was linked to increased $K_{ir}2.1$ and KCNQ1 channel expression, in addition to $K_{2P}3.1$ upregulation. Expression of the L-type calcium channel α subunit $Ca_v1.2$ was not significantly altered, suggesting that the reduction of $I_{Ca,L}$ is not caused primarily by downregulation of the expression of its α subunit.[2] Furthermore, there was significant downregulation of repolarizing K^+ channels

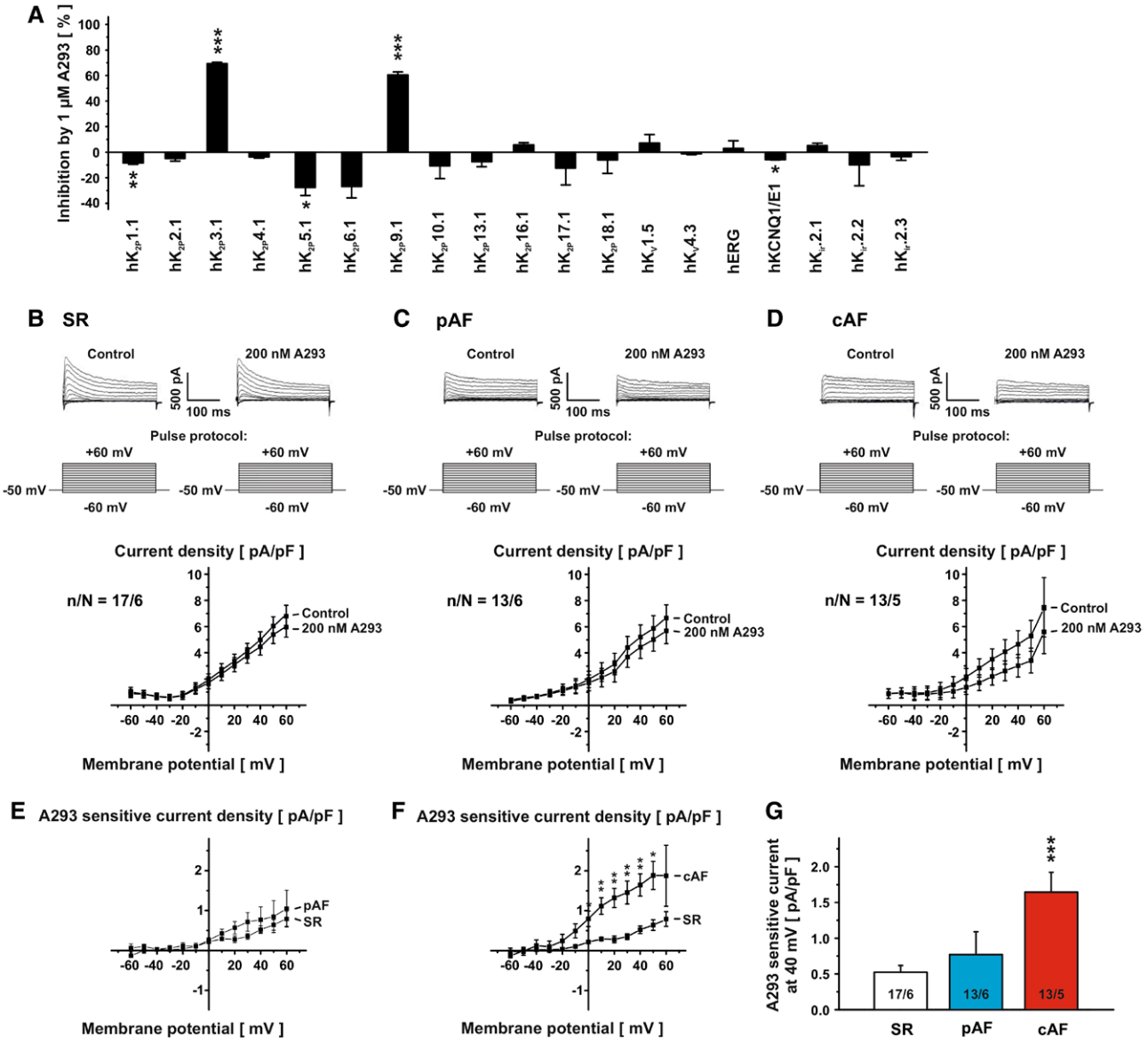

Figure 4. $K_{2P}3.1$ current properties in sinus rhythm (SR), paroxysmal atrial fibrillation (pAF), and chronic atrial fibrillation (cAF). **A**, Specificity of the $K_{2P}3.1$ inhibitor A293 assessed in *Xenopus* oocytes (n=4–14 cells were studied; see the online-only Data Supplement for details). Significant current reduction was observed with human $K_{2P}3.1$ and related, noncardiac $K_{2P}9.1$ channels. **B** through **D**, Representative macroscopic currents recorded from human right atrial myocytes using indicated voltage protocols and corresponding mean step current density as a function of the respective test potentials are displayed (**top** to **bottom**) for SR (**B**), pAF (**C**), and cAF (**D**). $K_{2P}3.1$ current was isolated with the use of the specific inhibitor A293. **E** and **F**, Current-voltage relationships of mean A293-sensitive current density obtained in **B** through **D** are depicted compared with SR for patients with pAF (**E**) and cAF (**F**). **G,** Mean A293-sensitive current density quantified at 40-mV membrane potential. Data are expressed as mean±SEM. n/N indicates number of myocytes/number of patients. **P*<0.05, ***P*<0.01, ****P*<0.001 vs drug-free control conditions (**A**) or vs SR (**E–G**).

($K_{ir}3.1$, $K_{ir}3.4$, $K_v4.3$), which is consistent with previous data and would prolong rather than shorten atrial APD. We conclude that $K_{2P}3.1$ upregulation, in combination with increased $K_{ir}2.1$ and KCNQ1 levels, accounts for APD shortening in patients with cAF. AF-related $K_{2P}3.1$ dysregulation and APD shortening strongly suggest a mechanistic role in cAF perpetuation with implications for patient-tailored antiarrhythmic therapy.

Therapeutic Implications: $K_{2P}3.1$ Inhibition Provides Mechanism-Based AF Management

Atrial selectivity is a desired target in the development of novel compounds for AF. Limiting the electropharmacological action to atrial tissue reduces the risk of proarrhythmic effects in the ventricles.[23] Inhibitors of $K_{2P}3.1$ channels, which are expressed predominantly in human atria and enhanced in AF, are therefore expected to be particularly effective and safe in AF therapy. In addition, the ability of an antiarrhythmic intervention to prevent AF depends on its capacity to suppress the underlying disease mechanism. Specifically, the reversal of atrial remodeling by targeting substrate development has become a focus of attempts at therapeutic intervention. The present study reveals $K_{2P}3.1$ current upregulation as a distinct arrhythmogenic substrate in cAF associated with abbreviated APD. Antiarrhythmic drugs with class III characteristics

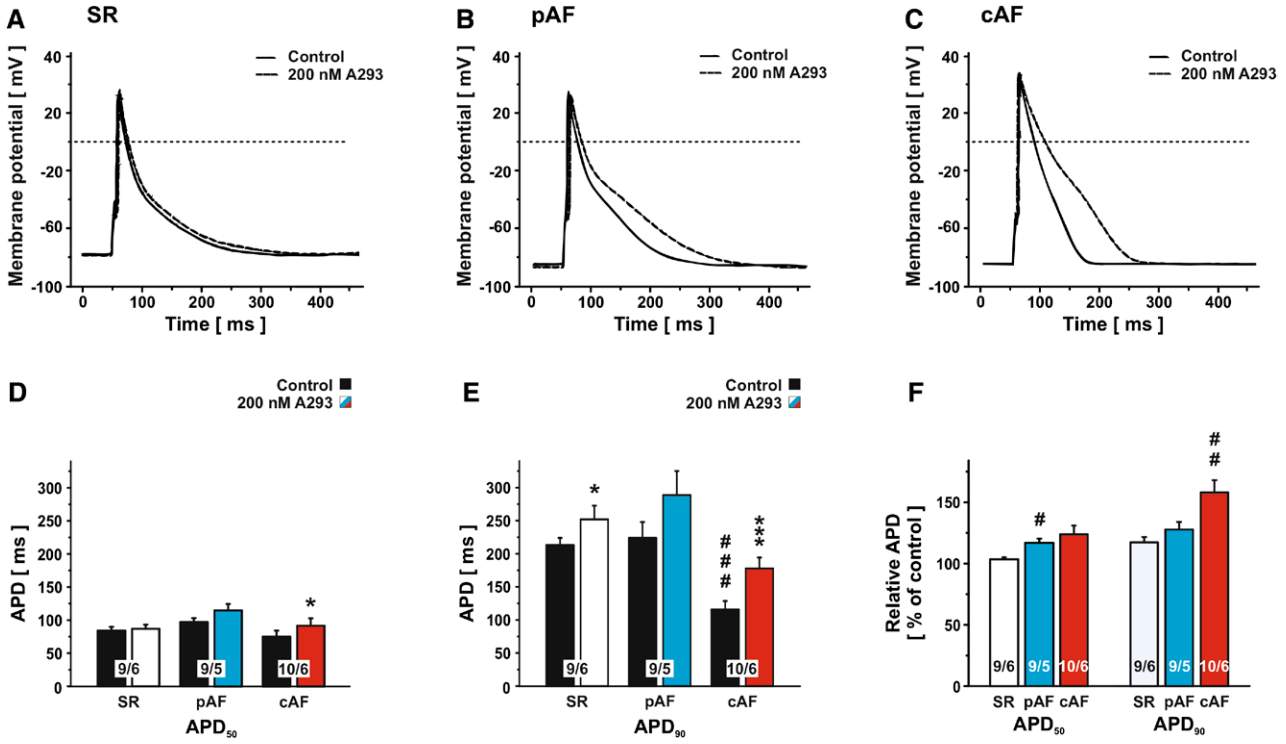

Figure 5. Characteristics of action potentials (APs) and electropharmacological effects of $K_{2P}3.1$ current blockade in right atrial myocytes. **A** through **C**, Representative APs recorded at 0.2 Hz in the absence or presence of A293 are shown for sinus rhythm (SR; **A**), paroxysmal atrial fibrillation (pAF; **B**), and chronic atrial fibrillation (cAF; **C**) patients. **D** and **E**, Corresponding mean AP durations at 50% of repolarization (APD_{50}; **D**) and 90% repolarization (APD_{90}; **E**) at baseline and after specific $K_{2P}3.1$ inhibition with 200 nmol/L A293. **F**, Relative APD_{50} and APD_{90} after application of 200 nmol/L A293 in atrial myocytes obtained from patients with indicated cardiac rhythm (values were normalized to respective baseline APD in the absence of A293). Data are provided as mean±SEM. n/N indicates number of myocytes/number of patients. **P*<0.05, ****P*<0.001 vs drug-free control conditions; #*P*<0.05, ##*P*<0.01, ###*P*<0.001 vs SR.

suppress AF through K^+ channel inhibition, resulting in prolongation of APD and prevention of electric re-entry. Here, specific $K_{2P}3.1$ inhibition by 200 nmol/L A293 prolonged the APD in patients with cAF to achieve levels observed in SR subjects, resulting in functional correction of electric remodeling in this AF subentity. Finally, diminished $K_{2P}3.1$ expression in AF subentities with severely reduced LVEF provides a criterion for personalized antiarrhythmic therapy: Clinical efficacy of $K_{2P}3.1$ inhibition is expected primarily in patients with cAF and normal or mildly to moderately reduced LVEF. Studies in large animals and humans are required next to further explore this novel antiarrhythmic paradigm in vivo.

Potential Limitations

AF-associated electric remodeling was studied in right and left atrial appendage tissue, revealing a previously unrecognized mechanism of AF pathophysiology. It remains unclear whether the results may be extrapolated to other atrial regions that have not been specifically assessed owing to the limited availability of these samples. Statistically significant $K_{2P}3.1$ upregulation was detected in right atrial tissue only (Figure 1). However, there was also a tendency toward increased $K_{2P}3.1$ mRNA levels in left atrial tissue obtained from patients with cAF that did not reach formal significance owing to a single outlier in the SR group. Thus, we suggest that $K_{2P}3.1$ enhancement is likely to occur in left atrial tissue as well, indicating that therapeutic interventions targeting $K_{2P}3.1$ upregulation in patients with cAF may be effective in both right and left atrial tissue.

Study patients were carefully matched for baseline characteristics, medication, and concomitant heart disease to exclude any bias associated with these conditions. In particular, no patient received class I or class III antiarrhythmic therapy that may have modulated APD. There were minor intergroup differences in age, cardiac function, cardiovascular disease, or medication as potential confounding factors that require consideration in the interpretation of our results. However, $K_{2P}3.1$ enhancement may not be attributed to impaired LVEF because we observed a correlation of severely reduced LV function with decreased rather than increased $K_{2P}3.1$ levels.

We did not investigate constitutive $I_{K,ACh}$ activity that was previously implicated in APD shortening. Given that selective $K_{2P}3.1$ inhibition by A293 in patients with cAF fully reconstituted APD, the contribution of constitutive $I_{K,ACh}$ activity to APD appears to be minor in the present subentity of patients with cAF. Unspecific antibody detection of cardiac protein observed in knockout mice requires consideration in the interpretation of human $K_{2P}3.1$ immunoblot data (Supplemental Results, Table III, and Figure III in the online-only Data Supplement).[19] We cannot fully exclude that available $K_{2P}3.1$ antibodies, including those used in this work, which were previously applied to demonstrate cardiac $K_{2P}3.1$ expression in mice, rats, dogs, and humans (Table III in the online-only Data Supplement), may recognize other proteins in humans as well. Therefore, the additional confirmation of increased $K_{2P}3.1$ expression at the protein level needs to be interpreted with caution. In human ventricular tissue, low protein levels

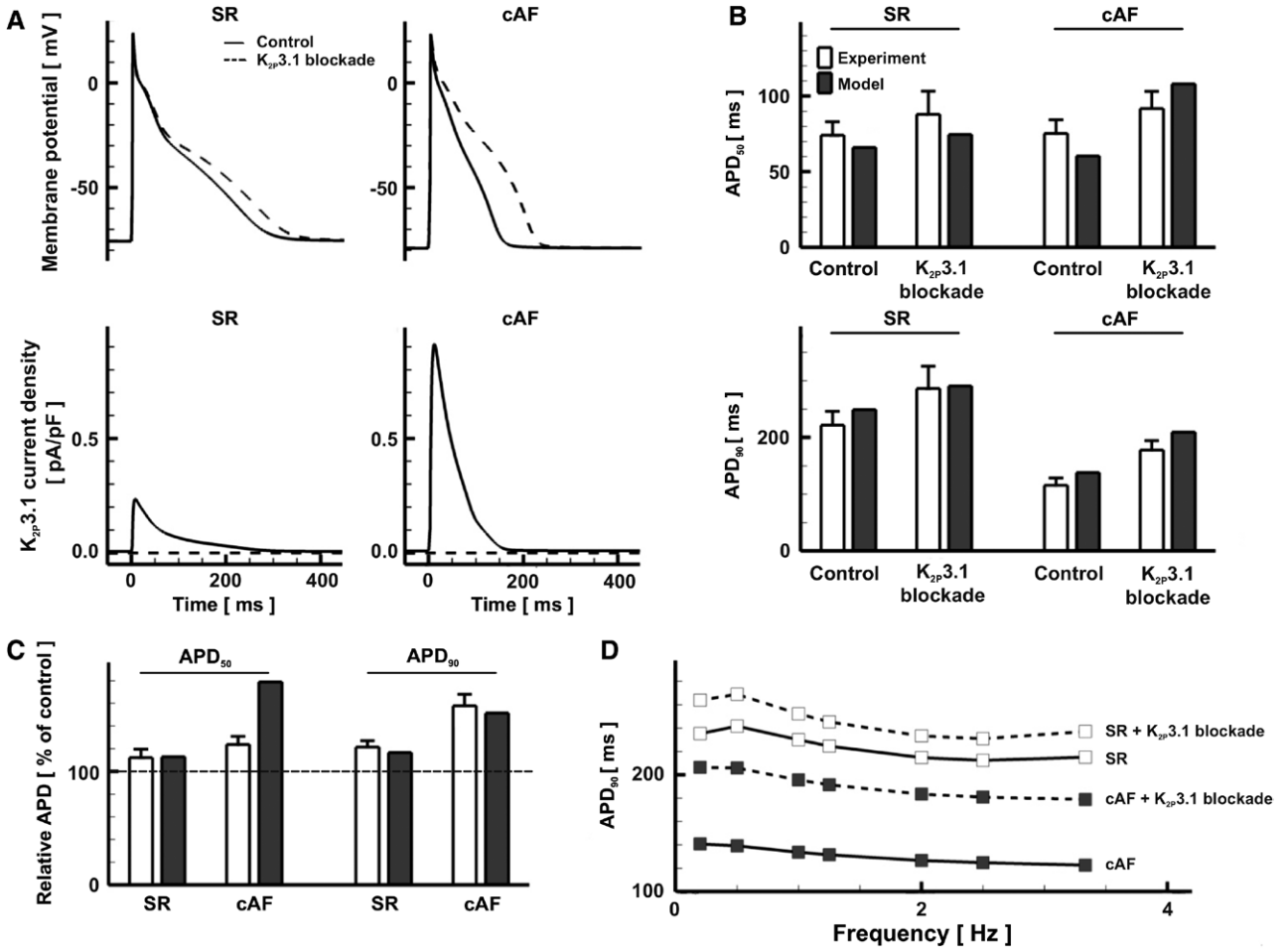

Figure 6. Computational analysis of the impact of K_{2P}3.1 channels on action potential duration (APD). **A**, Action potential (**top**) and K_{2P}3.1 current (**bottom**) in the sinus rhythm (SR; **left**) and chronic atrial fibrillation (cAF; **right**) models under control conditions (solid lines) or after complete inhibition of K_{2P}3.1 current (dashed lines). Data were obtained at a pacing frequency of 0.2 Hz with intracellular and extracellular ion concentrations based on the experimental pipette and bath solutions. **B**, Validation of APD at 50% of repolarization (APD_{50}; **top**) and APD at 90% (APD_{90}; **bottom**) in the SR and cAF models under control conditions and after K_{2P}3.1 blockade (solid bars) compared with measurements in isolated human atrial cardiomyocytes from patients with SR and cAF in the absence or presence of 200 nmol/L A293 (open bars). Experimental data are identical to those in Figure 5. **C**, Validation of the relative prolongation of APD_{50} and APD_{90} as a result of K_{2P}3.1 channel blockade based on the data from **B**. **D**, Rate dependence of APD prolongation after K_{2P}3.1 blockade in the SR (open symbols) and cAF models (solid symbols) with dynamic intracellular ion concentrations.

were detected by anti-K_{2P}3.1 antibodies, arguing against relevant cross-reactivity with endogenous human cardiac protein.

Altered ion channel transcript and protein levels analyzed in cardiac tissue may reflect alterations not only in myocytes but also in fibroblasts and other cell types. Importantly, in the present work, electrophysiological recordings provide unequivocal confirmation of K_{2P}3.1 current and APD remodeling in atrial myocytes.

Finally, structural alterations of atrial tissue may contribute to the development and maintenance of AF, in addition to electric remodeling.[1,2,31,33] Specifically, atrial fibrosis, which has been implicated in conduction heterogeneity and

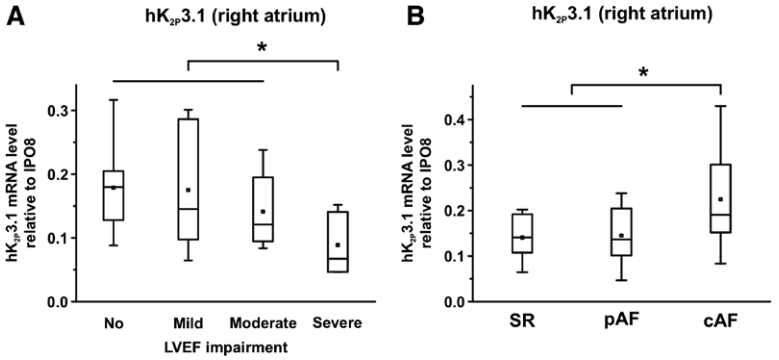

Figure 7. Correlation of right atrial K_{2P}3.1 mRNA levels with cardiac function. **A**, K_{2P}3.1 mRNA expression in subjects with normal left ventricular ejection fraction (LVEF) and with mildly, moderately, or severely impaired LVEF. **B**, Transcript levels in patients with sinus rhythm (SR), paroxysmal atrial fibrillation (pAF), and chronic atrial fibrillation (cAF). Data are expressed as mean±SEM arbitrary units normalized to importin 8 (IPO8). **P*<0.05 vs normal/mildly impaired/moderately reduced LVEF (**A**) or vs SR/pAF (**B**).

in the promotion of AF, is commonly observed in human AF and in animal models. Structural remodeling was not addressed here because the present study focused on the contribution of $K_{2P}3.1$ current dysregulation to electric remodeling only.

Conclusions

The data provide novel mechanistic insights into atrial arrhythmogenesis in humans. We detailed increased atrial $K_{2P}3.1$ expression and function in patients with cAF that resulted in shortening of AP recorded from patient-derived atrial myocytes. Specific $K_{2P}3.1$ inhibition prolonged APD in cardiac myocytes obtained from patients with cAF to reconstitute levels of SR subjects. Functional correction of atrial ionic remodeling through $K_{2P}3.1$ channel blockade represents a novel paradigm to optimize and specify AF management.

Acknowledgments

We thank Simone Bauer, Jennifer Gütermann, Bianca Stadler, Kai Sona, and Nadine Weiberg for excellent technical assistance, as well as the operating room team at the Department of Cardiac Surgery of Heidelberg University for supporting our work. We are grateful to Qiang Sun, Kathrin Kupser, Ramona Nagel, and Claudia Liebetrau (Division of Experimental Cardiology, Medical Faculty Mannheim, University of Heidelberg) for collegial support during the course of our study.

Sources of Funding

This study was supported in part by research grants from the University of Heidelberg, Faculty of Medicine (Rahel Goitein-Straus Scholarship and Olympia-Morata Scholarship to Dr Schmidt), from the DZHK (Deutsches Zentrum für Herz-Kreislauf-Forschung–German Center for Cardiovascular Research) through the BMBF (German Ministry of Education and Research; to Drs Katus, Dobrev, and Thomas), from the DFG (German Research Foundation; Do 769/1-3 to Dr Dobrev), from the Fondation Leducq (ENAFRA; to Dr Dobrev), from the European Union (European Network for Translational Research in Atrial Fibrillation, EUTRAF, grant 261057; to Dr Dobrev), from the German Cardiac Society and the Hengstberger Foundation (Klaus-Georg and Sigrid Hengstberger Scholarship to Dr Thomas), from the German Heart Foundation/German Foundation of Heart Research (F/08/14 to Dr Thomas), and from the Joachim Siebenreicher Foundation (to Dr Thomas). Dr Wiedmann was supported by the Otto-Hess-Scholarship of the German Cardiac Society, and Dr Baczkó was supported by the Hungarian National Development Agency cofinanced by the European Social Fund (TÁMOP-4.2.2.A-11/1/KONV-2012-0073 and 4.2.4.A/2-11/1-2012-0001 "National Program of Excellence").

Disclosures

The experimental compound A293 was kindly provided by Sanofi-Aventis (Frankfurt am Main, Germany). Dr Thomas served on advisory boards for and received honoraria for lectures from Sanofi-Aventis. The other authors report no conflicts.

References

1. Schmidt C, Kisselbach J, Schweizer PA, Katus HA, Thomas D. The pathology and treatment of cardiac arrhythmias: focus on atrial fibrillation. *Vasc Health Risk Manag*. 2011;7:193–202. doi: 10.2147/VHRM.S10758.
2. Schotten U, Verheule S, Kirchhof P, Goette A. Pathophysiological mechanisms of atrial fibrillation: a translational appraisal. *Physiol Rev*. 2011;91:265–325. doi: 10.1152/physrev.00031.2009.
3. Soucek R, Thomas D, Kelemen K, Bikou O, Seyler C, Voss F, Becker R, Koenen M, Katus HA, Bauer A. Genetic suppression of atrial fibrillation using a dominant-negative ether-a-go-go-related gene mutant. *Heart Rhythm*. 2012;9:265–272. doi: 10.1016/j.hrthm.2011.09.008.
4. Ravens U, Poulet C, Wettwer E, Knaut M. Atrial selectivity of antiarrhythmic drugs. *J Physiol*. 2013;591:4087–4097.
5. 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.
6. Dobrev D, Friedrich A, Voigt N, Jost N, Wettwer E, Christ T, Knaut M, Ravens U. The G protein-gated potassium current I(K,ACh) is constitutively active in patients with chronic atrial fibrillation. *Circulation*. 2005;112:3697–3706. doi: 10.1161/CIRCULATIONAHA.105.575332.
7. Gaborit N, Steenman M, Lamirault G, Le Meur N, Le Bouter S, Lande G, Léger J, Charpentier F, Christ T, Dobrev D, Escande D, Nattel S, Demolombe S. Human atrial ion channel and transporter subunit gene-expression remodeling associated with valvular heart disease and atrial fibrillation. *Circulation*. 2005;112:471–481. doi: 10.1161/CIRCULATIONAHA.104.506857.
8. Nattel S, Maguy A, Le Bouter S, Yeh YH. Arrhythmogenic ion-channel remodeling in the heart: heart failure, myocardial infarction, and atrial fibrillation. *Physiol Rev*. 2007;87:425–456. doi: 10.1152/physrev.00014.2006.
9. Thomas D, Plant LD, Wilkens CM, McCrossan ZA, Goldstein SA. Alternative translation initiation in rat brain yields K2P2.1 potassium channels permeable to sodium. *Neuron*. 2008;58:859–870. doi: 10.1016/j.neuron.2008.04.016.
10. Staudacher K, Baldea I, Kisselbach J, Staudacher I, Rahm AK, Schweizer PA, Becker R, Katus HA, Thomas D. Alternative splicing determines mRNA translation initiation and function of human $K_{2P}10.1$ K+ channels. *J Physiol*. 2011;589:3709–3720.
11. Gierten J, Ficker E, Bloehs R, Schlömer K, Kathöfer S, Scholz E, Zitron E, Kiesecker C, Bauer A, Becker R, Katus HA, Karle CA, Thomas D. Regulation of two-pore-domain (K2P) potassium leak channels by the tyrosine kinase inhibitor genistein. *Br J Pharmacol*. 2008;154:1680–1690. doi: 10.1038/bjp.2008.213.
12. Gierten J, Hassel D, Schweizer PA, Becker R, Katus HA, Thomas D. Identification and functional characterization of zebrafish K(2P)10.1 (TREK2) two-pore-domain K(+) channels. *Biochim Biophys Acta*. 2012;1818:33–41. doi: 10.1016/j.bbamem.2011.09.015.
13. Rahm AK, Gierten J, Kisselbach J, Staudacher I, Staudacher K, Schweizer PA, Becker R, Katus HA, Thomas D. PKC-dependent activation of human K(2P) 18.1 K(+) channels. *Br J Pharmacol*. 2012;166:764–773. doi: 10.1111/j.1476-5381.2011.01813.x.
14. Rahm AK, Wiedmann F, Gierten J, Schmidt C, Schweizer PA, Becker R, Katus HA, Thomas D. Functional characterization of zebrafish K2P18.1 (TRESK) two-pore-domain K+ channels. *Naunyn Schmiedebergs Arch Pharmacol*. 2014;387:291–300. doi: 10.1007/s00210-013-0945-1.
15. Seyler C, Duthil-Straub E, Zitron E, Gierten J, Scholz EP, Fink RH, Karle CA, Becker R, Katus HA, Thomas D. TASK1 (K(2P)3.1) K(+) channel inhibition by endothelin-1 is mediated through Rho kinase-dependent phosphorylation. *Br J Pharmacol*. 2012;165:1467–1475. doi: 10.1111/j.1476-5381.2011.01626.x.
16. Seyler C, Li J, Schweizer PA, Katus HA, Thomas D. Inhibition of cardiac two-pore-domain K+ (K2P) channels by the antiarrhythmic drug vernakalant: comparison with flecainide. *Eur J Pharmacol*. 2014;724:51–57. doi: 10.1016/j.ejphar.2013.12.030.
17. Putzke C, Wemhöner K, Sachse FB, Rinné S, Schlichthörl G, Li XT, Jaé L, Eckhardt I, Wischmeyer E, Wulf H, Preisig-Müller R, Daut J, Decher N. The acid-sensitive potassium channel TASK-1 in rat cardiac muscle. *Cardiovasc Res*. 2007;75:59–68. doi: 10.1016/j.cardiores.2007.02.025.
18. Decher N, Wemhöner K, Rinné S, Netter MF, Zuzarte M, Aller MI, Kaufmann SG, Li XT, Meuth SG, Daut J, Sachse FB, Maier SK. Knockout of the potassium channel TASK-1 leads to a prolonged QT interval and a disturbed QRS complex. *Cell Physiol Biochem*. 2011;28:77–86. doi: 10.1159/000331715.
19. Donner BC, Schullenberg M, Geduldig N, Hüning A, Mersmann J, Zacharowski K, Kovacevic A, Decking U, Aller MI, Schmidt KG. Functional role of TASK-1 in the heart: studies in TASK-1-deficient mice show prolonged cardiac repolarization and reduced heart rate variability. *Basic Res Cardiol*. 2011;106:75–87. doi: 10.1007/s00395-010-0128-x.
20. Petric S, Clasen L, van Wessel C, Geduldig N, Ding Z, Schullenberg M, Mersmann J, Zacharowski K, Aller MI, Schmidt KG, Donner BC. *In vivo* electrophysiological characterization of TASK-1 deficient mice. *Cell Physiol Biochem*. 2012;30:523–537. doi: 10.1159/000341435.

21. Liang B, Soka M, Christensen AH, Olesen MS, Larsen AP, Knop FK, Wang F, Nielsen JB, Andersen MN, Humphreys D, Mann SA, Huttner IG, Vandenberg JI, Svendsen JH, Haunsø S, Preiss T, Seebohm G, Olesen SP, Schmitt N, Fatkin D. Genetic variation in the two-pore domain potassium channel, TASK-1, may contribute to an atrial substrate for arrhythmogenesis. *J Mol Cell Cardiol*. 2014;67:69–76. doi: 10.1016/j.yjmcc.2013.12.014.
22. Schmidt C, Wiedmann F, Langer C, Tristram F, Anand P, Wenzel W, Lugenbiel P, Schweizer PA, Katus HA, Thomas D. Cloning, functional characterization, and remodeling of K2P3.1 (TASK-1) potassium channels in a porcine model of atrial fibrillation and heart failure. *Heart Rhythm*. 2014;11:1798–1805. doi: 10.1016/j.hrthm.2014.06.020.
23. Ravens U. Novel pharmacological approaches for antiarrhythmic therapy. *Naunyn Schmiedebergs Arch Pharmacol*. 2010;381:187–193. doi: 10.1007/s00210-009-0487-8.
24. Limberg SH, Netter MF, Rolfes C, Rinné S, Schlichthörl G, Zuzarte M, Vassiliou T, Moosdorf R, Wulf H, Daut J, Sachse FB, Decher N. TASK-1 channels may modulate action potential duration of human atrial cardiomyocytes. *Cell Physiol Biochem*. 2011;28:613–624. doi: 10.1159/000335757.
25. Gierten J, Ficker E, Bloehs R, Schweizer PA, Zitron E, Scholz E, Karle C, Katus HA, Thomas D. The human cardiac K2P3.1 (TASK-1) potassium leak channel is a molecular target for the class III antiarrhythmic drug amiodarone. *Naunyn Schmiedebergs Arch Pharmacol*. 2010;381:261–270. doi: 10.1007/s00210-009-0454-4.
26. Staudacher K, Staudacher I, Ficker E, Seyler C, Gierten J, Kisselbach J, Rahm AK, Trappe K, Schweizer PA, Becker R, Katus HA, Thomas D. Carvedilol targets human K2P 3.1 (TASK1) K+ leak channels. *Br J Pharmacol*. 2011;163:1099–1110. doi: 10.1111/j.1476-5381.2011.01319.x.
27. Schmidt C, Wiedmann F, Schweizer PA, Becker R, Katus HA, Thomas D. Novel electrophysiological properties of dronedarone: inhibition of human cardiac two-pore-domain potassium (K2P) channels. *Naunyn Schmiedebergs Arch Pharmacol*. 2012;385:1003–1016. doi: 10.1007/s00210-012-0780-9.
28. Schmidt C, Wiedmann F, Schweizer PA, Becker R, Katus HA, Thomas D. Class I antiarrhythmic drugs inhibit human cardiac two-pore-domain K(+) (K2 $_2$p) channels. *Eur J Pharmacol*. 2013;721:237–248. doi: 10.1016/j.ejphar.2013.09.029.
29. Schmidt C, Wiedmann F, Schweizer PA, Katus HA, Thomas D. Inhibition of cardiac two-pore-domain K+ (K2P) channels: an emerging antiarrhythmic concept. *Eur J Pharmacol*. 2014;738:250–255. doi: 10.1016/j.ejphar.2014.05.056.
30. Bikou O, Thomas D, Trappe K, Lugenbiel P, Kelemen K, Koch M, Soucek R, Voss F, Becker R, Katus HA, Bauer A. Connexin 43 gene therapy prevents persistent atrial fibrillation in a porcine model. *Cardiovasc Res*. 2011;92:218–225. doi: 10.1093/cvr/cvr209.
31. Trappe K, Thomas D, Bikou O, Kelemen K, Lugenbiel P, Voss F, Becker R, Katus HA, Bauer A. Suppression of persistent atrial fibrillation by genetic knockdown of caspase 3: a pre-clinical pilot study. *Eur Heart J*. 2013;34:147–157. doi: 10.1093/eurheartj/ehr269.
32. Lugenbiel P, Thomas D, Kelemen K, Trappe K, Bikou O, Schweizer PA, Voss F, Becker R, Katus HA, Bauer A. Genetic suppression of Gαs protein provides rate control in atrial fibrillation. *Basic Res Cardiol*. 2012;107:1–12.
33. Schmidt C, Wiedmann F, Tristram F, Anand P, Wenzel W, Lugenbiel P, Schweizer PA, Katus HA, Thomas D. Cardiac expression and atrial fibrillation-associated remodeling of K_2p2.1 (TREK-1) K^+ channels in a porcine model. *Life Sci*. 2014;97:107–115. doi: 10.1016/j.lfs.2013.12.006.
34. Karle CA, Zitron E, Zhang W, Wendt-Nordahl G, Kathöfer S, Thomas D, Gut B, Scholz E, Vahl CF, Katus HA, Kiehn J. Human cardiac inwardly-rectifying K+ channel Kir(2.1b) is inhibited by direct protein kinase C-dependent regulation in human isolated cardiomyocytes and in an expression system. *Circulation*. 2002;106:1493–1499.
35. Voigt N, Li N, Wang Q, Wang W, Trafford AW, Abu-Taha I, Sun Q, Wieland T, Ravens U, Nattel S, Wehrens XH, Dobrev D. Enhanced sarcoplasmic reticulum Ca_2^+ leak and increased Na^+-Ca_2^+ exchanger function underlie delayed afterdepolarizations in patients with chronic atrial fibrillation. *Circulation*. 2012;125:2059–2070. doi: 10.1161/CIRCULATIONAHA.111.067306.
36. Grandi E, Pandit SV, Voigt N, Workman AJ, Dobrev D, Jalife J, Bers DM. Human atrial action potential and Ca_2^+ model: sinus rhythm and chronic atrial fibrillation. *Circ Res*. 2011;109:1055–1066. doi: 10.1161/CIRCRESAHA.111.253955.
37. Voigt N, Heijman J, Trausch A, Mintert-Jancke E, Pott L, Ravens U, Dobrev D. Impaired Na^+-dependent regulation of acetylcholine-activated inward-rectifier K^+ current modulates action potential rate dependence in patients with chronic atrial fibrillation. *J Mol Cell Cardiol*. 2013;61:142–152. doi: 10.1016/j.yjmcc.2013.03.011.
38. Voigt N, Heijman J, Wang Q, Chiang DY, Li N, Karck M, Wehrens XH, 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.
39. Voigt N, Zhou XB, Dobrev D. Isolation of human atrial myocytes for simultaneous measurements of Ca_2^+ transients and membrane currents. *J Vis Exp*. 2013;77:e50235.
40. Christ T, Wettwer E, Voigt N, Hála O, Radicke S, Matschke K, Várro A, Dobrev D, Ravens U. Pathology-specific effects of the IKur/Ito/IK,ACh blocker AVE0118 on ion channels in human chronic atrial fibrillation. *Br J Pharmacol*. 2008;154:1619–1630. doi: 10.1038/bjp.2008.209.
41. Girmatsion Z, Biliczki P, Bonauer A, Wimmer-Greinecker G, Scherer M, Moritz A, Bukowska A, Goette A, Nattel S, Hohnloser SH, Ehrlich JR. Changes in microRNA-1 expression and IK1 up-regulation in human atrial fibrillation. *Heart Rhythm*. 2009;6:1802–1809. doi: 10.1016/j.hrthm.2009.08.035.
42. Voigt N, Trausch A, Knaut M, Matschke K, Varró A, Van Wagoner DR, Nattel S, Ravens U, Dobrev D. Left-to-right atrial inward rectifier potassium current gradients in patients with paroxysmal versus chronic atrial fibrillation. *Circ Arrhythm Electrophysiol*. 2010;3:472–480. doi: 10.1161/CIRCEP.110.954636.

CLINICAL PERSPECTIVE

Mechanism-based approaches to atrial fibrillation (AF) therapy are sought to increase effectiveness and to provide more individualized patient care. Specifically, the reversal of atrial remodeling by targeting substrate development has become a focus of attempts at therapeutic intervention. Shortening of atrial refractory periods promotes electric re-entry and contributes to maintenance of AF. Outward currents mediated by K_{2P}3.1 (TASK-1) 2-pore-domain potassium (K_{2P}) channels promote repolarization and have been implicated in action potential (AP) regulation in animal models. Their functional contribution to atrial electrophysiology in patients with AF, however, is not known. The present work provides novel mechanistic insights into atrial arrhythmogenesis in humans. Cellular electrophysiology, molecular biology, biochemistry, and computational modeling were used to assess the significance of K_{2P}3.1 channels and their remodeling in patients with paroxysmal and persistent, long-standing persistent, or permanent (chronic) AF compared with subjects in sinus rhythm. K_{2P}3.1 subunits exhibited predominant atrial expression. We observed increased K_{2P}3.1 expression and function in patients with chronic AF that resulted in shortening of AP duration in patient-derived atrial myocytes. In patients with paroxysmal AF, K_{2P}3.1 levels were not significantly affected, in line with a lack of AP duration changes. Pharmacological K_{2P}3.1 inhibition prolonged AP duration in cardiac myocytes obtained from patients with chronic AF to reconstitute levels of subjects in sinus rhythm. This work provides the first direct evidence of K_{2P}3.1 dysregulation resulting in AP duration shortening in patients with chronic AF, suggesting a mechanistic role of K_{2P}3.1 in chronic AF perpetuation. Functional correction of atrial ionic remodeling through K_{2P}3.1 blockade represents a novel paradigm to optimize and specify AF management.