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

Constance 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 Borggreve, MD; Peter Biliczki, MD, PhD; Joachim R. Ehrlich, MD; István Baczkó, MD, PhD; Patrick Luguenbiel, 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

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, FHIRS, Department of Cardiology, University of Heidelberg, Im Neuenheimer Feld 410, D-69120 Heidelberg, Germany. E-mail dierk.thomas@med.uni-heidelberg.de

© 2015 American Heart Association, Inc.

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.⁴ Although constitutive Iₖ,CAₐ₅ activity, increased I,K⁺ current, and decreased I,CAₐ₅ have previously been implicated in APD shortening during AF, the contribution of other ion channels is poorly understood.⁵⁻⁸

Two-pore-domain K⁺ (K₂P) channels facilitate AP repolarization, and regulation of K₂P current dynamically determines cellular excitability.⁹⁻¹⁰ Specifically, cardiac K₂P3.1 (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 K₂P3.1 channels results in APD prolongation in rodents.¹⁷⁻²⁰ In the human heart, K₂P3.1 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 K₂P3.1 channels as drug targets is further supported by their sensitivity to established antiarrhythmic compounds.²⁵⁻²⁸ The aim of this study was to explore the potential contribution of K₂P3.1 current dysregulation to AF-related APD abbreviation and to assess the relevance of K₂P3.1 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 K₂P3.1 (1:200; APC-024; Alomone Labs, Jerusalem, Israel), as described.⁹⁻³³ 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.¹³ The K₂P3.1 channel inhibitor A293 (2-[butan-1-sulfonylamino]-N-[1-(R)-(6-methoxy-pyridin-3-yl)-propyl]-benzamidê)¹⁷ (kindly provided by Sanofi-Aventis, Berlin, Germany) was applied to isolate K₂P3.1 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.¹⁶ computational model of the human atrial cardiomyocyte, including our recent update with Na⁺-dependent regulation of I,K⁺ and I,CA₂,₅,²⁹ was extended with a formulation for the K₂P3.1 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 (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

K₂P Channel Expression in the Human Heart

A comprehensive expression analysis of all human K₂P isoforms identified K₂P1.1 and K₂P3.1 as predominant K₂P subunits in the right and left atria of patients with SR (n=14; Figure 1). K₂P3.1 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⁺ channels (Figure 1). In LV tissue samples (n=5), K₂P3.1 transcript levels were low compared with the right atrium (16-fold; n=5–10; P<0.001) 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 K₂P3.1 mRNA expression was similar to K,4.3 channels conducting the cardiac transient outward K⁺ current and to inward-rectifying potassium channels K,2.2 and K,2.3 (Figure 2).

Increased K₂P3.1 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 K₂P3.1 mRNA expression in the right atrium was elevated by 59.8%
(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 K2P3.1 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, K2P3.1 mRNA levels did not change in patients with pAF (n=16) compared with patients in SR (n=14; Figure 1). Alterations of K2P3.1 mRNA expression levels were consistent with K2P3.1 immunoblots (Figure 3 and Figure II in the online-only Data Supplement). cAF was associated with upregulation of K2P3.1 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 K2P3.1 protein expression in pAF (37.4±13.1%; P=0.043; n=4). Of note, K2P3.1 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-K2P3.1 antibodies in an exemplary ventricular sample, highlighting weak K2P3.1 expression in LV tissue (Figure IIIA in the online-only Data Supplement). However, limited discrimination of K2P3.1 and other cardiac proteins by anti-K2P3.1 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).

### Table. Baseline Characteristics of Study Patients

<table>
<thead>
<tr>
<th></th>
<th>RAA</th>
<th>LAA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SR</td>
<td>pAF</td>
</tr>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men, n (%)</td>
<td>25</td>
<td>19</td>
</tr>
<tr>
<td>Age, y</td>
<td>63.7±13.8</td>
<td>71.7±12.6*</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.1±4.8</td>
<td>27.9±4.6</td>
</tr>
<tr>
<td>Height, cm</td>
<td>170±9.6</td>
<td>171±10.7</td>
</tr>
<tr>
<td>Medical history, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAD</td>
<td>24</td>
<td>21</td>
</tr>
<tr>
<td>AVD</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>MVD</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CAD+AVD</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Hypertension</td>
<td>34</td>
<td>28</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>LVEF, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>21</td>
<td>15</td>
</tr>
<tr>
<td>Mild reduced</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Moderate reduced</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Severe reduced</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Concomitant medication, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digitalis</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>22</td>
<td>16</td>
</tr>
<tr>
<td>AT1 antagonists</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>Diuretics</td>
<td>13</td>
<td>20</td>
</tr>
<tr>
<td>Nitrates</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Lipid-lowering drugs</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>OAC</td>
<td>9</td>
<td>22</td>
</tr>
</tbody>
</table>

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.

Downloaded from http://ahajournals.org by on July 22, 2019
channels and accessory subunits relevant to atrial electrophysiology (Figure 2). Of note, we did not detect significant electric remodeling in patients with pAF.

**K$_{\text{p3.1}}$ Current Enhancement in cAF**

Functional consequences of K$_{\text{p3.1}}$ upregulation were studied in right atrial myocytes obtained from patients with SR, pAF, and cAF. K$_{\text{p3.1}}$ 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). A293-sensitive K$^+$ currents activated at potentials $\geq$−20 mV and showed Goldman-Hodgkin-Katz (open or outward) rectification that is characteristic of K$_{\text{2P}}$ channels (Figure 4B–4F).

K$_{\text{p3.1}}$ 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$_{\text{p3.1}}$ 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$_{\text{p3.1}}$ Upregulation Is Associated With APD Shortening**

Upregulation of K$_{\text{p3.1}}$ 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$_{\text{90}}$) 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$_{\text{p3.1}}$ currents. In patients with pAF (n/N=9/5), APD$_{\text{90}}$ 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$_{\text{50}}$; Figure 5A–5D) or resting membrane potential (Figure V in the online-only Data Supplement) in any group.

**Class III Antiarrhythmic Effects of K$_{\text{p3.1}}$ Channel Inhibition in cAF Patients**

The experimental K$_{\text{p3.1}}$ inhibitor A293 was used to test the hypothesis that pharmacological K$_{\text{p3.1}}$ reduction would reverse APD shortening in cAF. In human atrial myocytes obtained from patients in SR (n/N=9/6), K$_{\text{p3.1}}$ block by 200 nmol/L A293 induced only a weak prolongation of APD$_{\text{90}}$ (3.4±1.6%; $P$=0.11) and APD$_{\text{50}}$ (17.1±4.5%; $P$=0.012; Figure 5A and 5D–5F). In contrast, APD$_{\text{90}}$ 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$_{\text{50}}$ in pAF, albeit to a lesser degree (27.8±6.3%; $P$=0.003; Figure 5B, 5E, and 5F). A direct
comparison of the A293 effects between patients with SR, pAF, and cAF revealed that specific K2P3.1 blockade had little effect on absolute APD90 in SR and pAF (Figure 5E), whereas in patients with cAF, A293 increased APD90 to APD levels typical for SR subjects (Figure 5E and 5F).

Computational Analysis of the Effect of K2P3.1 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 K2P3.1 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 APD50 and APD90 under simulated conditions corresponding to the experimentally used pipette and bath solutions (Figure 6A and 6B). Simulated inhibition of K2P3.1 channels produced a modest prolongation of APD90 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 K2P3.1 channel blockade approached that of the SR model, with a reduction in the APD difference from 93.2 to 28.1 milliseconds (~70%) after K2P3.1 channel blockade compared with SR simulations. Together, these data suggest that, under these conditions, upregulation of K2P3.1 in patients with cAF plays a major role in the proarrhythmic APD shortening.
The analysis revealed a significant association between LV function and cardiac rhythm ($F=11.8; P=0.35; \text{Kendall } \tau=-0.16$) in the patient cohort.

### Discussion

**Atrial $K_{\text{r3.1}}$ K$^+$ Channels in Humans With SR**

$K_{\text{r3.1}}$ 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 $K_{\text{r3.1}}$ channels in left and right atrium obtained from control subjects with SR. $K_{\text{r3.1}}$ displayed highest transcript levels among $K_{\text{r3.1}}$ family members with confirmed K$^+$ channel function (ie, after exclusion of $K_{\text{r3.1}1}$, $K_{\text{r3.1}7.1}$, $K_{\text{r3.1}12.1}$, and $K_{\text{r3.1}15.1}$ subunits, which do not produce substantial K$^+$ currents) and was specifically studied. The high ratio of atrial to ventricular $K_{\text{r3.1}}$ transcripts (16:1) highlighted predominantly atrial expression. Inhibition of $K_{\text{r3.1}}$ current produced a tendency toward prolonged APD$_{90}$ by 17% in patients in SR, reflecting class III antiarrhythmic effects. These data indicate that $K_{\text{r3.1}}$ 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 $K_{\text{r3.1}}$ 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.\(^3\) 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 $K_{\text{r3.1}}$ current had no effect on resting membrane potential. The molecular basis of electric remodeling was further elucidated in a comprehensive approach that included all $K_{\text{r3.1}}$ channels and 21 additional ion channel subunits relevant to atrial electrophysiology. The main finding was a significant upregulation of $K_{\text{r3.1}}$ 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 nonactivating outward K$^+$ currents in patient-derived atrial myocytes after extensive pharmacological block of established potassium channels additionally highlights a significant contribution of $K_{\text{r3.1}}$ conductance to human cardiac electrophysiology.\(^40\)

AF-associated APD shortening has previously been attributed to increased $I_{\text{K1}}$ current, downregulation of $I_{\text{Ca,L}}$, and constitutively active $I_{\text{K,acch}}$ (despite decreased $K_{\text{r3.1}}$ and $K_{\text{r3.4}}$ subunits underlying the current).\(^3,41,42\) In the present cAF cohort, APD abbreviation was linked to increased $K_{\text{r3.1}}$ and KCNQ1 channel expression, in addition to $K_{\text{r3.1}}$ upregulation. Expression of the L-type calcium channel $\alpha$ subunit Ca.1.2 was not significantly altered, suggesting that the reduction of $I_{\text{Ca,L}}$ is not caused primarily by downregulation of the expression of its $\alpha$ subunit.\(^2\) Furthermore, there was significant downregulation of repolarizing K$^+$ channels

---

**Figure 3.** Western blot analysis of $K_{\text{r3.1}}$ 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-$K_{\text{r3.1}}$ antibodies. B. Anti-GAPDH antibodies were applied to quantify protein load. C. Means±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 $K_{\text{r3.1}}$ Expression**

To provide a more precise characterization of the patient population likely to benefit from $K_{\text{r3.1}}$ blockade, the correlation of right atrial $K_{\text{r3.1}}$ 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 $K_{\text{r3.1}}$ 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 $K_{\text{r3.1}}$ 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 ($F=11.8; P=0.35; \text{Kendall } \tau=-0.16$) in the patient cohort.

---

**APD Shortening in cAF Patients: Significance of $K_{\text{r3.1}}$ 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.\(^3\) 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 $K_{\text{r3.1}}$ current had no effect on resting membrane potential. The molecular basis of electric remodeling was further elucidated in a comprehensive approach that included all $K_{\text{r3.1}}$ channels and 21 additional ion channel subunits relevant to atrial electrophysiology. The main finding was a significant upregulation of $K_{\text{r3.1}}$ 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 nonactivating outward K$^+$ currents in patient-derived atrial myocytes after extensive pharmacological block of established potassium channels additionally highlights a significant contribution of $K_{\text{r3.1}}$ conductance to human cardiac electrophysiology.\(^40\)

AF-associated APD shortening has previously been attributed to increased $I_{\text{K1}}$ current, downregulation of $I_{\text{Ca,L}}$, and constitutively active $I_{\text{K,acch}}$ (despite decreased $K_{\text{r3.1}}$ and $K_{\text{r3.4}}$ subunits underlying the current).\(^3,41,42\) In the present cAF cohort, APD abbreviation was linked to increased $K_{\text{r3.1}}$ and KCNQ1 channel expression, in addition to $K_{\text{r3.1}}$ upregulation. Expression of the L-type calcium channel $\alpha$ subunit Ca.1.2 was not significantly altered, suggesting that the reduction of $I_{\text{Ca,L}}$ is not caused primarily by downregulation of the expression of its $\alpha$ subunit.\(^2\) Furthermore, there was significant downregulation of repolarizing K$^+$ channels
(K₃.1, K₃.4, K₄.3), which is consistent with previous data and would prolong rather than shorten atrial APD. We conclude that K₂P₃.1 upregulation, in combination with increased Kir2.1 and KCNQ1 levels, accounts for APD shortening in patients with cAF. AF-related K₂P₃.1 dysregulation and APD shortening strongly suggest a mechanistic role in cAF perpetuation with implications for patient-tailored antiarrhythmic therapy.

**Therapeutic Implications: K₂P₃.1 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. Inhibitors of K₂P₃.1 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 K₂P₃.1 current upregulation as a distinct arrhythmogenic substrate in cAF associated with abbreviated APD. Antiarrhythmic drugs with class III characteristics

![Figure 4.](image)
suppress AF through K⁺ channel inhibition, resulting in prolongation of APD and prevention of electric re-entry. Here, specific K₂P3.1 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 K₂P3.1 expression in AF subentities with severely reduced LVEF provides a criterion for personalized antiarrhythmic therapy: Clinical efficacy of K₂P3.1 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 K₂P3.1 upregulation was detected in right atrial tissue only (Figure 1). However, there was also a tendency toward increased K₂P3.1 mRNA levels in left atrial tissue obtained from 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.

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, K₂P3.1 enhancement may not be attributed to impaired LVEF because we observed a correlation of severely reduced LV function with decreased rather than increased K₂P3.1 levels.

We did not investigate constitutive I_KACh activity that was previously implicated in APD shortening. Given that selective K₂P3.1 inhibition by A293 in patients with cAF fully reconstituted APD, the contribution of constitutive I_KACh 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 K₂P3.1 immunoblot data (Supplemental Results, Table III, and Figure III in the online-only Data Supplement).19 We cannot fully exclude that available K₂P3.1 antibodies, including those used in this work, which were previously applied to demonstrate cardiac K₂P3.1 expression in mice, rats, and 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 K₂P3.1 expression at the protein level needs to be interpreted with caution. In human ventricular tissue, low protein levels...
were detected by anti-K_{p}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_{p}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.\textsuperscript{1,2,31,33} Specifically, atrial fibrosis, which has been implicated in conduction heterogeneity and
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 Güterbann, Bianca Stadler, Kai Son, 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 Liebetrat (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 Ministry for 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 (ENAFA; 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 Hamburgerst Berger Foundation (Klaus-Georg and Sigrid Hamburger 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**


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) potassium channels promote 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 atrial fibrillation. Cellular and molecular mechanisms of atrial arrhythmogenicity were visualized in an atrial cardiomyocyte model. In vivo animal models were used to investigate the effects of K_2P_3.1 channel blockade on atrial fibrillation. These studies provide a novel paradigm for AF management.