Repolarization Alternans Reveals Vulnerability to Human Atrial Fibrillation

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Background—The substrates for human atrial fibrillation (AF) are poorly understood, but involve abnormal repolarization (action potential duration [APD]). We hypothesized that beat-to-beat oscillations in APD may explain AF substrates, and why vulnerability to AF forms a spectrum from control subjects without AF to patients with paroxysmal then persistent AF.

Methods and Results—In 33 subjects (12 with persistent AF, 13 with paroxysmal AF, and 8 controls without AF), we recorded left (n=33) and right (n=6) atrial APD on pacing from cycle lengths 600 to 500 ms (100 to 120 bpm) up to the point where AF initiated. Action potential duration alternans required progressively faster rates for patients with persistent AF, patients with paroxysmal AF, and controls (cycle length 411±94 versus 372±72 versus 218±33 ms; P<0.01). In AF patients, APD alternans occurred at rates as slow as 100 to 120 bpm, unrelated to APD restitution (P>0.10). In this milieu, spontaneous ectopy initiated AF. At fast rates, APD alternans disorganized to complex oscillations en route to AF. Complex oscillations also arose at progressively faster rates for persistent AF, paroxysmal AF, and controls (cycle length: 316±99 versus 266±19 versus 177±16 ms; P=0.02). In paroxysmal AF, APD oscillations amplified before AF (P<0.001). In controls, APD alternans arose only at very fast rates (cycle length <250 ms; P<0.001 versus AF groups) just preceding AF. In 4 AF patients in whom rapid pacing did not initiate AF, APD alternans arose transiently then extinguished.

Conclusions—Atrial APD alternans reveals dynamic substrates for AF, arising most readily (at lower rates and higher magnitudes) in persistent AF then paroxysmal AF, and least readily in controls. APD alternans preceded all AF episodes and was absent when AF did not initiate. The cellular mechanisms for APD alternans near resting heart rates require definition. (Circulation. 2011;123:2922-2930.)

Key Words: —atrium ■ fibrillation ■ action potentials ■ electrophysiology ■ remodeling

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We hypothesized that the remodeled atria of patients with persistent AF would exhibit APD alternans at slow heart rates, and that progressively faster rates would be required to elicit APD alternans in patients with paroxysmal AF or controls without AF. We tested this hypothesis by studying left and right atrial APD oscillations vis-à-vis AF initiation during incremental pacing from near-resting heart rates in patients with and without AF.

Methods

Patient Flow
We prospectively enrolled 33 patients referred for ablation to the Veterans Administration and the University of California Medical Centers in San Diego, CA, 25 for ablation of AF (12 persistent) and 8 controls (6 with accessory pathways, 1 with atrial tachycardia, and 1 with premature ventricular complexes) without AF. We approached for enrollment all consecutive patients undergoing AF ablation between December 2005 and March 2009, when research staff and catheters were available (n=54), excluding those with decompensated heart failure or coronary disease, and we report

Clinical Perspective on p 2930

Alternans of action potential duration (APD) creates a milieu of repolarization dispersion that, in animal ventricles and in silico, may cause fibrillation directly or by interacting with slow conduction or ectopy.4–6 In humans, right atrial APD alternans7,8 explains AF transitions from typical atrial flutter9 or pacing,8 whereas left atrial APD alternans may explain AF initiation from pulmonary vein ectopy.10 However, atrial APD alternans has typically been reported only at fast rates because of APD-rate dependence (restitution)1,5 or altered cellular calcium handling,5,11 and it is unclear whether alternans differs between AF patients and individuals without AF without AF.
Pacing Protocol
Patients in AF were electrically cardioverted to sinus rhythm and studied after 15 minutes, preceding ablation. Action potentials were recorded from the distal poles of the MAP catheter while pacing from the proximal poles or a nearby stable position. Pacing was delivered for 74 beats at cycle lengths (CL) 500 ms, 450 ms, 400 ms, 350 ms, and 300 ms, then in 10 ms steps to AF or capture failure (n=6), whichever came first. In 5 patients, pacing started at 600 ms, 550 ms, then the above sequence.

Signal filtering was 0.05 to 500 Hz (APs), 30 to 500 Hz (other intracardiac signals), and 0.05 to 100 Hz (ECG). Signals were digitized at 1 kHz to 16-bit resolution (Bard Pro, Billerica, MA) and exported for analysis using software written in Labview (National Instruments, Austin, TX).

Measurement of Action Potential Duration
We measured APD using validated methods6,10,13–16 with manual verification. We assigned AP onset as the time of maximal computed upstroke dV/dt and determined phase II voltage and phase IV (diastolic) voltage in the 5 ms preceding AP upstroke (Figure 2A). Action potential duration90 was measured from AP onset to 90% voltage recovery from phase II. Diastolic interval (DI) spans from APD90 of the prior beat to AP onset.10

Measurement of Action Potential Duration Restitution
We constructed curves of APD restitution during pacing using ≥5 (DI, APD90) pairs at each pacing CL. Maximum slope was determined from linear fits for the shortest 30-ms DI segment with data (eg, 0 to 30 ms or 10 to 40 ms) as previously described.10

Measurement of Action Potential Duration Alternans and Complex Oscillations
We measured pairwise differences in APD90 (ΔAPD, Figure 2A), summarized by mean absolute ΔAPD for the last 10 beats at each CL. Alternans was assigned if ΔAPD alternated in polarity with magnitude ≥5% of mean APD90 (baseline APD varies ±2%7,9). Because APD alternans may disorganize to complex oscillations preceding arrhythmia onset17,18 via phase reversals (eg, Long-Short-Long-Short-Long (LSLLS) proceeding to LSLSS; Figure 2) or AP shape changes (Figure 3), we also report mean absolute ΔAPD for 10 nonalternating beats (if ≥5% mean APD).

Statistical Analysis
Continuous data are represented as mean±SD. ANOVA was used to compare variables among 3 patient groups, such as APD alternans magnitude or onset CL, with posthoc Tukey-Kramer tests to identify differences between group pairs. Paired continuous variables, such as the DI of APD alternans and APD restitution slope, were compared using linear regression and the paired t test. Group differences in APD90 at selected CLs were evaluated with separate ANOVAs using a Bonferroni correction for testing multiple CL bins. Action potential duration restitution slope was also compared between groups using a mixed-effects model with an unstructured covariance matrix that incorporated all observations with DIs in the range 0–30 ms.

Table 1. Clinical Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Persistent AF (n=12)</th>
<th>Paroxysmal AF (n=13)</th>
<th>Controls (n=8)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>65±11†</td>
<td>64±8†</td>
<td>48±15</td>
<td>0.010</td>
</tr>
<tr>
<td>Gender, M, F</td>
<td>12.0</td>
<td>12.1</td>
<td>6.2</td>
<td>0.16</td>
</tr>
<tr>
<td>Duration of AF, mo</td>
<td>71±72</td>
<td>64±132</td>
<td>. . .</td>
<td>0.87</td>
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<tr>
<td>Left atrial diameter, mm</td>
<td>47±5†</td>
<td>40±5</td>
<td>36±2</td>
<td>&lt;0.001</td>
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<tr>
<td>LV ejection fraction, %</td>
<td>54±11</td>
<td>60±7</td>
<td>62±8</td>
<td>0.19</td>
</tr>
<tr>
<td>NYHA Class I/II</td>
<td>11</td>
<td>13</td>
<td>6</td>
<td>0.41</td>
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<tr>
<td>NYHA Class III</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.71</td>
</tr>
<tr>
<td>Coronary Disease, n (%)</td>
<td>3 (27)</td>
<td>5 (46)</td>
<td>1 (13)</td>
<td>0.42</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Medications, n</th>
<th>ACEI/ARB</th>
<th>Statins</th>
<th>β-blockers</th>
<th>Class I agents</th>
<th>Amiodarone</th>
<th>Sotalol</th>
<th>Dofetilide</th>
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<tbody>
<tr>
<td></td>
<td>6†</td>
<td>6</td>
<td>9±2</td>
<td>1</td>
<td>1</td>
<td>1±1†</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>8†</td>
<td>9</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>6†</td>
<td>0</td>
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<td></td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>2±0†</td>
</tr>
</tbody>
</table>

AF indicates atrial fibrillation; NYHA, New York Heart Association functional class; ACEI, angiotensin-converting enzyme inhibitors; ARB, aldosterone receptor blockers.

*P<0.05 vs paroxysmal AF.
†P<0.05 vs controls.
ms < DI < 100ms. This model adjusts for correlated observations within individuals. A log transform of DI was applied to account for the nonlinear relationship over this broader DI range. The Fisher exact test was applied to contingency tables. Biatrial data were primarily analyzed; right versus left atrial data are presented separately. \( P < 0.05 \) was considered statistically significant.

**Results**

Our patient sample is described in Table 1. Patients with persistent AF had larger left atria than those with paroxysmal AF or controls. We performed 39 incremental pacing experiments in left (\( n = 33 \)) and right (\( n = 6 \)) atria, recording their transitions to AF.

**Action Potential Duration Alternans Preceded Atrial Fibrillation Transitions in Different Patterns Between Groups**

Action potential duration alternans preceded every AF initiation and arose at slower rates (ie, more easily) in patients with persistent AF than in those with paroxysmal AF and controls (Table 2). With continued incremental pacing, APD alternans transitioned to complex oscillations (Figures 2 through 4), also at slower rates in persistent AF than in paroxysmal AF and controls (ANOVA \( P = 0.03 \)). Sinus rhythm rates did not differ between groups.

**Figure 2.** Action potential duration alternans at slow rates in persistent AF, disorganizing to complex oscillations just preceding AF. This man (left atrium diameter 44 mm, left ventricular ejection fraction 44%) showed substantial APD alternans at 500 ms (A). CL 450 ms (mean APD≈120 ms for 10 beats), (B) CL 400 ms during intermittent AV block, with a phase reversal (?), (C) CL 280 ms with multiple phase reversals, and (D) CL 280 ms, with complex oscillations then AF. E, Maximum APD restitution slope <1, RSPV indicates right superior pulmonary vein; MAP, monophasic action potential; CSmid, mid coronary sinus; Ph II, phase 2; CL, cycle length; S, short; L, long; A, atrial signal; V, ventricular signal; AV, atrioventricular; and AF, atrial fibrillation.

**Figure 3.** Intermediate-rate alternans in paroxysmal AF, with rate-dependent onset of complex oscillations preceding AF. This 61-year-old man (left atrial diameter 42 mm, left ventricular ejection fraction 65%), showed (A) No APD alternans at CL 500 ms, (B) APD alternans at CL 320 ms (LSL…), during 4:1, 2:1 AV ratios, which (C) exaggerated at CL 280 ms, with complex oscillations abruptly before AF onset. D, Action potential duration restitution had maximum slope >1 but was <1 at time of APD alternans onset. LSPV indicates left superior pulmonary vein; MAP, monophasic action potential; CSmid, mid coronary sinus; CL, cycle length; S, short; L, long; A, atrial signal; V, ventricular signal; AV, atrioventricular; and AF, atrial fibrillation.
In a patient with persistent AF, Figure 2 shows left atrial APD alternans at slow rates that disorganized at faster rates to complex APD oscillations en route to AF. At CL 500 ms (120 bpm), APD alternans had magnitude 58 ms (22% of mean APD), increasing to 120 ms (44% of APD 272 ms, Figure 2A) at CL 450 ms (130 bpm) that continued at CL 400 ms (150 bpm) during atrioventricular (AV) block (21% of APD; phase reversals marked * in Figure 2B) and CL 290 ms (207 bpm; 20% of APD, Figure 2C). At CL 210 ms (286 bpm), complex APD oscillations led directly to AF (Figure 2D).

In patients with paroxysmal AF, Figures 3 and 4 show APD alternans at intermediate rates en route to AF. In Figure 3A, pacing CL 500 ms (120 bpm) showed APD 309 ± 8 ms without alternans (< 5% of APD). At CL 320 ms (188 bpm), APD alternans had magnitude 25 ms during 4:1 and 2:1 AV conduction (10% of APD 243 ms; Figure 3B), increasing at CL 280 ms (214 bpm) to 67 ms (34% of APD 198 ms; Figure 3C) immediately preceding AF. Figure 4 shows no APD alternans at CL 500 ms (120 bpm, Figure 4A), small amplitude APD alternans at CL 300 ms (200 bpm, Figure 4B) that increased at CL 250 ms (240 bpm) to magnitude 14 ms

Table 2. Repolarization Dynamics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Persistent AF</th>
<th>Paroxysmal AF</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Segments, LA/RA</td>
<td>12/3</td>
<td>13/2</td>
<td>8/1</td>
<td>0.81</td>
</tr>
<tr>
<td>Sinus CL, ms</td>
<td>997 ± 378</td>
<td>1067 ± 215</td>
<td>890 ± 73</td>
<td>0.40</td>
</tr>
<tr>
<td>CL of AF Initiation, ms</td>
<td>208 ± 31</td>
<td>232 ± 35</td>
<td>200 ± 25</td>
<td>0.068</td>
</tr>
<tr>
<td>Max S1-rest slope</td>
<td>1.1 ± 0.5</td>
<td>1.2 ± 0.5</td>
<td>0.8 ± 0.2</td>
<td>0.078</td>
</tr>
<tr>
<td>APD alternans</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Onset CL, ms</td>
<td>411 ± 94†</td>
<td>372 ± 72†</td>
<td>218 ± 30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean APD at onset, ms</td>
<td>223 ± 51</td>
<td>260 ± 37†</td>
<td>181 ± 24</td>
<td>0.008</td>
</tr>
<tr>
<td>DI (pre-shorter APD), ms</td>
<td>162 ± 79†</td>
<td>100 ± 48†</td>
<td>29 ± 12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>APDR slope at onset DI</td>
<td>0.29 ± 0.17</td>
<td>0.35 ± 0.15</td>
<td>0.44 ± 0.10</td>
<td>0.11</td>
</tr>
<tr>
<td>Magnitude at onset, ms</td>
<td>24 ± 13</td>
<td>23 ± 13</td>
<td>13 ± 5</td>
<td>0.16</td>
</tr>
<tr>
<td>Magnitude at onset, % APD</td>
<td>11 ± 5</td>
<td>9 ± 5</td>
<td>8 ± 4</td>
<td>0.41</td>
</tr>
<tr>
<td>Complex APD oscillations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset CL, ms</td>
<td>316 ± 99†</td>
<td>266 ± 19</td>
<td>177 ± 16</td>
<td>0.017</td>
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<tr>
<td>APD at onset, ms</td>
<td>205 ± 49</td>
<td>204 ± 29</td>
<td>194 ± 65</td>
<td>0.90</td>
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<tr>
<td>Magnitude, ms</td>
<td>23 ± 24</td>
<td>18 ± 26</td>
<td>7 ± 6</td>
<td>0.43</td>
</tr>
<tr>
<td>Magnitude, % APD</td>
<td>10 ± 9</td>
<td>9 ± 12</td>
<td>4 ± 4</td>
<td>0.56</td>
</tr>
</tbody>
</table>

AF indicates atrial fibrillation; LA, left atrium; RA, right atrium; CL, cycle lengths; Max, maximum; APD, action potential duration; and DI, diastolic interval.

*P<0.05 vs paroxysmal AF.
†P<0.05 vs controls.

Figure 4. Amplification of rate-dependent APD alternans in paroxysmal AF preceding AF. This patient (left atrial diameter 39 mm, left ventricular ejection fraction 59%) showed (A) no APD alternans at CL 500 ms, (B) APD alternans at CL 300 ms, (C) marked APD alternans at CL 250 ms (phase II/III shape) during 3:1 AV conduction preceding AF initiation. Asterisk indicates far-field AF activation or an after-depolarization triggering AF. D. Action potential duration restitution slope was >1 at its maximum but <1 at APD alternans onset. RA indicates right atrium; MAP, monophasic action potential; CSmid, mid coronary sinus; CL, cycle length; S, short; L, long; A, atrial signal; V, ventricular signal; AV, atrioventricular; and AF, atrial fibrillation.
(7% of APD=213 ms) during 3:1 AV conduction just preceding AF. The small MAP signal (asterisk) preceding AF may indicate a far-field signal or an after-depolarization.

Although paroxysmal AF patients typically present in sinus rhythm and persistent AF patients in AF, 2 paroxysmal AF patients presenting in AF developed APD alternans at CL=400 ms and 300 ms that increased with rate just before AF. One persistent AF patient presented in sinus rhythm with APD alternans at CL 500 ms (7% of mean APD) transitioning via complex oscillations to AF.

Notably, control subjects showed APD alternans of small magnitude (5 ms, 8% of APD), developing only at very rapid rates (CL 218±30 ms, **P**<0.001 against either AF group) just before AF. Figure 5 shows a control patient in whom APD alternans was absent at CL between 500 ms and CL 250 ms. Alternans developed at CL 240 ms (250 bpm) at small magnitude (5% of mean APD) during 2:1 and 4:1 AV ratios, then increased at CL 200 ms (300 bpm; 8% of APD) immediately before AF. No control subject had APD alternans at CL ≥250 ms (**P**<0.001 versus either AF group).

**Onset of Action Potential Duration Alternans Did Not Correlate With Action Potential Duration Restitution Slope or Atrioventricular Conduction**

Action potential duration restitution did not differ significantly between groups when analyzed for maximum slope (**Table 2**) or by a mixed-effects model for the log-linear relationship (**P**=0.15). Maximum restitution slope was >1 in some patients (Figures 3D and 4D) and <1 in others (Figure 2E). Importantly, at the onset of APD alternans in AF patients, APD restitution slope was always <1 (slope 0.8 in Figure 2; 0.5 at CL 300 ms in Figure 3D; 0.4 at CL 300 ms in Figure 4D) even if maximum slope was >1 at faster rates. In controls, APD alternans arose both when restitution slope was near/above 1 (eg, Figure 5E) and <1 (Table 2).

Onset CL for APD alternans differed from onset CL of 2:1 AV conduction (**P**<0.001, paired *t* test), and was longer than onset CL of 2:1 AV conduction by 79±72 ms (persistent AF), 72±62 ms (paroxysmal AF), and 18±25 ms (controls). Each of Figures 2 through 5 shows APD alternans when 2:1 AV conduction was present and absent.

**Rate Dependence of Action Potential Duration Oscillations**

Figure 6A shows the atrial APD-CL curve (Figure 6A) for each group. In AF patients, APD was shorter for those with persistent than paroxysmal AF at CL 500 ms and 400 ms (**P**<0.0125, using Bonferroni corrections) but not CL 300 ms or 200 ms.

Notably, the CL of onset and CL range for which APD oscillations were observed differed markedly between groups. Figure 6B illustrates the magnitude of APD oscillations (pair-wise APD range over 10 beats, centered at APD mean) for each CL. Action potential duration oscillations spanned all rates in persistent AF (red envelope) and paroxysmal AF (blue envelope), but occurred only at fast rates in controls (green envelope). In 3 AF patients paced at 100 bpm (CL 600 ms; not plotted), APD oscillations occurred with magnitude 13±10% of mean APD=297±74 ms. Considering only patients whose APD alternans arose without 2:1 AV conduction, alternans onset CL was 444±80 ms (persis-
tent AF), 418±59 (paroxysmal AF), and 245±49 (controls; P=0.004, ANOVA).

Figure 6C summarizes the magnitude of APD oscillations (as a percentage of APD) against CL for each group. Persistent AF exhibited APD oscillations for all rates, whereas paroxysmal AF exhibited rate-dependent increases in APD oscillations (P<0.001). Controls showed small magnitude APD alternans only at very rapid rates. Relative to AF initiation, patients with paroxysmal AF showed amplified APD oscillations as CL shortened to AF initiation (P<0.001); patients with persistent AF showed a weak negative relationship (P<0.05).

APD Dynamics vis-à-vis Atrial Fibrillation Vulnerability and Atrial Fibrillation Initiation
APD oscillations separated patients at different stages of remodeling (Figure 6). Unlike controls, AF patients showed APD oscillations >5% of APD between 120 bpm (CL 500 ms) and 240 bpm (CL 250 ms). Paroxysmal AF patients differed from persistent AF patients by amplification in APD oscillations as CL shortened to AF initiation (P<0.001); patients with persistent AF showed a weak negative relationship (P<0.05).

Action potential duration oscillations led to AF via 2 mechanisms. Figure 7A illustrates APD alternans at slow rates (CL 450 ms illustrated; also at CL 500 ms) when a spontaneous premature atrial complex (asterisked) triggered AF. Of note, APD restitution from single extra-systoles in this patient (S2-restitution) had maximum slope (1.79). Figures 2 through 5 show transitions to AF after amplified APD alternans (Figures 3 through 5) or via complex APD oscillations (Figure 2).

Discussion
This study shows that alternans of APD in human left and right atria indicate progressive substrates for, and susceptibility to, AF. We observed APD alternans of large amplitude near resting rate in patients with persistent AF, at intermediate amplitude and rates in patients with paroxysmal AF, and of small amplitude only at very rapid rates (>230 bpm) just before AF was induced in control subjects. Action potential duration oscillations preceded all transitions to AF. In patients with APD alternans at slow rates, ectopy initiated AF.

Rapid pacing failed to initiate AF in 4 patients. All patients initially exhibited atrial APD alternans, although 2:1 atrial capture then intervened to suppress alternans. This is shown for a persistent AF patient with APD restitution slope <1 (Figure 7B) but was also seen in paroxysmal AF and in patients with maximum APD restitution slope >1.

Right Versus Left Atrial Action Potential Duration Dynamics
Quantitatively, there were no differences between atria in APD alternans onset CL (P=0.81) or magnitude as a percentage of APD (P=0.26). Qualitatively, APD alternans in the right atrium was also marked at slow rates in AF patients. For instance, at CL 450 ms, APD alternans had a range of 1.8 to 120 ms (0.6% to 44% of APD) in left atrium and 8.2 to 32 ms (3.4% to 14% of APD) in right atrium. Biatrial APD statistics were similar when right atrial data were excluded from analysis.
Differential Onset and Dynamics of Action Potential Duration Alternans

The magnitude and onset rate of APD alternans paralleled the progressive susceptibility to AF observed in control subjects and in patients with paroxysmal and persistent AF. In control subjects, APD alternans occurred only at rapid rates, agreeing with a vast literature of computational and animal studies. However, marked APD alternans in AF patients near resting heart rates (Figure 2, summarized in Figure 6) stands in sharp contrast and points to potentially important differences in AF between humans and prior experimental models. At rapid rates, APD alternans may be explained by the restitution hypothesis. Restitution is the relationship of APD to rate, and a maximum slope $\geq 1$ leads to APD alternans at rapid rates and wavebreak. However, we found no overall relation between the onset rate of atrial APD alternans and restitution slope, as also reported in human ventricles. Thus, although APD restitution slope $\geq 1$ may explain AF initiation from ectopy in selected patients with paroxysmal AF (Figure 7A and prior work) or control subjects (Figure 5), it cannot explain APD alternans at slow rates in AF patients (when APD restitution is flat).

Cellular calcium overload is also a potential mechanism for APD alternans in the atrium, given that it has been shown to lower the onset rate of APD alternans in remodeled versus control animal and human ventricles. In animal models, diminished L-type calcium current and abnormal intracellular calcium handling from electric remodeling causes calcium overload. Human atrial myocytes also exhibit altered calcium handling, which explains AF in computational models and may explain reduced right atrial APD alternans by verapamil. Nevertheless, direct human evidence for calcium abnormalities as a cause for APD alternans or AF is lacking.

Atrial APD alternans may also be explained by electric remodeling of membrane ion currents. In canine atria, remodeling elevates potassium currents, compounding the effects of calcium overload to shorten the effective refractory period and compress APD range. Myocytes from remodeled human atria also exhibit increased inward repolarizing currents that shorten APD. This study confirms in vivo that left atrial APD has a flattened rate-response in patients with persistent AF compared with paroxysmal AF or controls (Figure 6A and Table 2). However, because APD shortening lengthens DI, it remains unclear how this explains APD alternans at slow rates.

Finally, atrial conduction slowing may theoretically cause APD alternans and transitions to AF and is a feature of structural remodeling. As expected, structural remodeling was more evident in patients with persistent than paroxysmal AF (Table 1). However, we recently showed that AF patients show broad left atrial conduction restitution (slowing) for premature beats only for DIs $\leq 50$ to $100$ ms. Thus, conduction restitution may not plausibly explain APD alternans at the longer DIs in this study (Table 2).

Action Potential Duration Alternans and Onset of Fibrillation

Action potential duration alternans preceded AF initiation across a wide range of rates and may provide a clinical index of AF susceptibility. These data are the first to characterize biastral APD dynamics en route to clinical AF and suggest that APD alternans may also be mechanistically involved in transitions to AF.

In animal ventricles and in silico, beat-to-beat repolarization dispersion (alternans) may cause wavebreak and fibrillation after ectopic beats or directly. First, during APD alternans at slow rates, spontaneous premature atrial complexes were observed to trigger AF (Figure 7A). In Figure 4, the small MAP deflection after a long APD (asterisked) may potentially indicate an after-depolarization induced by APD alternans that triggers AF, although further mapping is required to exclude far-field AF signals or noise (although these signals are relatively noise free). Second, APD altern-
ans at fast rates in AF patients transitioned to AF via amplified alternans or complex oscillations. This may represent period multiplying in nonlinear systems,17,18 which heralds ventricular fibrillation in canine ventricles18 or human ECG T-waves.27,28 Complex oscillations are consistent with the findings of Mironov et al,29 who found nodal lines during APD alternans that spanned the heart to cause phase reversals, reduce APD variability, and herald fibrillation. Higher spatial resolution mapping is required to confirm whether complex APD oscillations represent nodal lines within the MAP field of view.

Clinical Implications
First, APD oscillations detected invasively (eg, from implanted device leads) or potentially from the ECG30 may provide a tool of dynamic susceptibility to AF, to monitor the efficacy of therapy, or to identify patients’ risk of progression from paroxysmal to persistent AF.31 Second, therapy to prevent APD alternans, as recently shown for sarcoplasmonic endoplasmic reticulum Ca²⁺-ATPase gene therapy in guinea pig ventricles,32 may reduce AF vulnerability. Christini et al showed that ventricular APD alternans can be controlled clinically,33 which, with our findings, opens the possibility of preventing AF initiation. Indeed, when APD alternans was suppressed in the present study, AF was noninducible by rapid pacing (Figure 7B).

Limitations
A limitation of this study is that multiple sites were studied in only 6 patients because of the time required to pace into AF, cardiovert, wait 15 minutes, reposition the MAP catheter, and repeat the protocol. For this reason, we have limited statistical power to separate right and left atrial APD dynamics. Second, our sample sizes were small, largely because of the lack of availability of MAP catheters, although this remains the largest study of human left atrial APD dynamics to date. Control subjects were younger than AF patients, because patients aged 60 to 65 years who required left-sided access but did not have AF nor flutter were relatively uncommon and difficult to recruit. Third, although we focus on pacing-induced AF, this mimics atrial tachycardias that trigger AF and reliably initiates AF without the impracticalities of waiting for spontaneous AF. Moreover, we also observed spontaneous premature atrial complex–triggered AF (Figure 7A). Fourth, although pacing may distort end-repolarization, APD alternans onset occurred at slow enough rates for pacing to fall after APD (at least of the short beat, Figures 2A, 2B, 2B, 3C, 3B, and 4B). At faster rates, alternans often affected AP shape (Figures 2, 3C, and 4C), and its detection therefore remained robust. Fifth, the presence (persistent AF) or absence (paroxysmal AF) of AF just preceding the protocol may influence group differences. One persistent AF patient presenting in sinus rhythm and 2 paroxysmal AF patients presenting in AF displayed APD alternans in accordance with their groups. Sixth, we cannot categorically exclude an effect of aging, because it was challenging to enroll older patients with clinical indications for left-sided mapping without AF/Flutter. Nevertheless, 3 controls (aged 67, 64, and 69 years) showed APD dynamics that were similar to those of younger controls and distinct from AF patients. Seventh, statistically, although posthoc analyses were used after omnibus ANOVA tests, no adjustments were made for testing multiple variables. Eighth, our study had few women, reflecting our Veterans Affairs patients. Although sex differences in AF are unclear, studies in both genders are required.

Conclusions
Action potential duration alternans reveals a spectrum of substrates for and susceptibility to clinical AF. Control subjects and patients with paroxysmal and persistent AF showed progressive abnormalities in the onset rate, magnitude, and rate-response of APD alternans, in tandem with easier transitions to AF. When present at slow rates, APD alternans enabled ectopy to initiate AF, and at faster rates, APD alternans amplified or disorganized directly to AF. Cellular studies are required to explain the mechanisms enabling AF patients to exhibit APD oscillations at near-resting heart rates.

Sources of Funding
This study was supported in part by grants from the National Institutes of Health (HL70529 and HL83359) and the Doris Duke Charitable Foundation (to Dr Narayan).

Disclosures
Dr Narayan reports having received honoraria from Medtronic, St. Jude Medical, and Biotronik and grant support from Biosense-Webster. His Institution has received fellowship support from Medtronic, Boston Scientific, St. Jude Medical, and Biotronik. He is also the author of intellectual property owned by the University of California Regents and licensed to Topera Inc. Topera does not sponsor any research, including that presented here. Dr Franz was the original inventor of the MAP catheter used in this study. Dr Krummen has received honoraria from Medtronic. His Institution has received fellowship support from Medtronic, Boston Scientific, St. Jude Medical, and Biotronik. The other authors report no conflicts.

References
Human atrial fibrillation (AF) is a highly prevalent disease whose mechanisms are poorly understood. Ectopic beats from the pulmonary veins may trigger AF, but this likely also requires substrate, because ectopy rarely triggers AF in control subjects. We hypothesized that beat-to-beat oscillations in atrial repolarization (action potential duration [APD]) may provide an AF substrate and explain the spectrum of AF vulnerability from subjects without AF to those with paroxysmal AF and control subjects, in whom alternans developed only at very rapid rates (<100 bpm) in persistent AF patients, a finding that is difficult to explain by current theories and animal experiments. Action potential duration alternans onset required progressively faster rates for patients with near-resting heart rates (100 to 120 bpm) in persistent AF patients, a finding that is difficult to explain by current theories and animal experiments.

**CLINICAL PERSPECTIVE**

Human atrial fibrillation (AF) is a highly prevalent disease whose mechanisms are poorly understood. Ectopic beats from the pulmonary veins may trigger AF, but this likely also requires substrate, because ectopy rarely triggers AF in control subjects. We hypothesized that beat-to-beat oscillations in atrial repolarization (action potential duration [APD]) may provide an AF substrate and explain the spectrum of AF vulnerability from subjects without AF to those with paroxysmal and persistent AF. We found that APD alternans invariably preceded AF initiation. Notably, APD alternans arose at near-resting heart rates (100 to 120 bpm) in persistent AF patients, a finding that is difficult to explain by current theories and animal experiments. Action potential duration alternans onset required progressively faster rates for patients with paroxysmal AF and control subjects, in whom alternans developed only at very rapid rates (>230 bpm) just preceding induced AF. Furthermore, in patients in whom rapid pacing failed to initiate AF, APD alternans did not develop. In conclusion, APD alternans indicates dynamic substrates for AF and arises most readily in patients with persistent AF and least readily in control subjects without AF. Action potential duration alternans preceded every AF episode, yet was absent when AF was not induced. Accordingly, APD alternans provides a clinical tool to identify AF vulnerability and may be useful in refining diagnosis or monitoring the effectiveness of AF therapy.
Repolarization Alternans Reveals Vulnerability to Human Atrial Fibrillation
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Circulation. 2011;123:2922-2930; originally published online June 6, 2011; doi: 10.1161/CIRCULATIONAHA.110.977827
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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