Spectral Analysis Identifies Sites of High-Frequency Activity Maintaining Atrial Fibrillation in Humans

Prashanthan Sanders, MBBS, PhD*; Omer Berenfeld, PhD*; Mélèze Hocini, MD; Pierre Jaïs, MD; Ravi Vaidyanathan, BE; Li-Fern Hsu, MBBS; Stéphane Garrigue, MD, PhD; Yoshihide Takahashi, MD; Martin Rotter, MD; Frédéric Sacher, MD; Christophe Scavée, MD; Robert Ploutz-Snyder, PhD; José Jalife, MD; Michel Haïssaguerre, MD

Background—The identification of sites of dominant activation frequency during atrial fibrillation (AF) in humans and the effect of ablation at these sites have not been reported.

Methods and Results—Thirty-two patients undergoing AF ablation (19 paroxysmal, 13 permanent) during ongoing arrhythmia were studied. Electroanatomic mapping was performed, acquiring 126±13 points per patient throughout both atria and coronary sinus. At each point, 5-second electrograms were obtained to determine the highest-amplitude frequency on spectral analysis and to construct 3D dominant frequency (DF) maps. The temporal stability of the recording interval was confirmed in a subset. Ablation was performed with the operator blinded to the DF maps. The effect of ablation at sites with or without high-frequency DF sites (maximal frequencies surrounded by a decreasing frequency gradient ≥20%) was evaluated by determining the change in AF cycle length (AFCL) and the termination and inducibility of AF. The spatial distribution of the DF sites was different in patients with paroxysmal and permanent AF; paroxysmal AF patients were more likely to harbor the DF site within the pulmonary vein, whereas in permanent AF, atrial DF sites were more prevalent. Ablation at a DF site resulted in significant prolongation of the AFCL (180±30 to 198±40 ms; P<0.0001; k=0.77), whereas in the absence of a DF site, there was no change in AFCL (169±22 to 170±22 ms; P=0.4). AF terminated during ablation in 17 of 19 patients with paroxysmal and 0 of 13 with permanent AF (P<0.0001). When 2 patients with nonsustained AF during mapping were excluded, 13 of 15 (87%) had AF termination at DF sites (54% at the initially ablated DF site): 11 pulmonary veins and 2 atrial. In addition, AF could no longer be induced in 69% with termination of AF at a DF site. There were no significant differences in the number or percentage of DF sites detected (5.4±1.6 versus 4.9±2.1; P=0.3) and ablated (1.9±1.0 versus 2.4±1.0; P=0.3) in those with and without AF termination. The duration of radiofrequency ablation to achieve termination was significantly shorter than that delivered in those with persisting AF (34.8±24.0 versus 73.5±22.9 minutes; P=0.0002). All patients with persisting AF had additional DF sites outside the ablated zones.

Conclusions—Spectral analysis and frequency mapping identify localized sites of high-frequency activity during AF in humans with different distributions in paroxysmal and permanent AF. Ablation at these sites results in prolongation of the AFCL and termination of paroxysmal AF, indicating their role in the maintenance of AF. (Circulation. 2005;112:789-797.)

Key Words: atrium ■ mapping ■ fibrillation ■ remodeling ■ ablation

The widely accepted multiple-wavelet hypothesis of atrial fibrillation (AF) postulates that AF is sustained by numerous coexisting wave fronts of electrical activity that propagate randomly throughout the fibrillating atria. However, emerging experimental evidence suggests that certain cases of AF are maintained by small reentrant sources (rotors) that result in a hierarchical distribution of frequencies throughout the atria. Whether such rotors have a role in the maintenance of AF in humans is unknown.

The present study was designed to evaluate the feasibility of spectral analysis and dominant frequency (DF) mapping in patients with AF. By comparing the spatial distribution of DFs during AF with ablation outcome, we aimed to determine the role of regions of high-frequency activity in the maintenance of AF.

Methods

Study Population
The study comprised 32 patients with symptomatic drug-refractory AF undergoing ablation during ongoing arrhythmia. AF was paroxysmal in 19 and permanent in 13 patients. Baseline characteristics are presented in Table 1. Antiarrhythmics, except for amiodarone, were ceased ≥5 half-lives before the study. All patients gave written consent.

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*Drs Sanders and Berenfeld contributed equally to this article.
Correspondence to José Jalife, SUNY Upstate Medical University, 766 Irving Ave, Syracuse, NY 13210. E-mail jalifej@upstate.edu
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TABLE 1. Patient Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Paroxysmal AF</th>
<th>Permanent AF</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y (n=19)</td>
<td>55.7±9.3</td>
<td>58.0±6.8</td>
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</tr>
<tr>
<td>Male, n (%) (n=20)</td>
<td>16 (84)</td>
<td>11 (85)</td>
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</tr>
<tr>
<td>History of AF, mo</td>
<td>99.2±55.4</td>
<td>62.8±34.4</td>
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<tr>
<td>Sustained AF duration</td>
<td>3.4±2.9 h</td>
<td>18.4±16.6 mo</td>
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</tr>
<tr>
<td>LA size, mm</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Parasternal</td>
<td>42.1±6.9</td>
<td>50.3±7.1</td>
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</tr>
<tr>
<td>Longitudinal</td>
<td>50.3±10.4</td>
<td>63.8±10.7</td>
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</tr>
<tr>
<td>Transverse</td>
<td>35.3±8.2</td>
<td>45.5±6.3</td>
<td>0.002</td>
</tr>
<tr>
<td>LV dimension, mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV end-diastolic</td>
<td>54.7±5.7</td>
<td>55.3±4.7</td>
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</tr>
<tr>
<td>LV end-systolic</td>
<td>34.9±4.2</td>
<td>37.8±7.8</td>
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<tr>
<td>LV ejection fraction</td>
<td>68.0±3.5</td>
<td>57.3±17.5</td>
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<tr>
<td>Structural heart disease, n (%)</td>
<td>4 (21)</td>
<td>6 (46)</td>
<td>0.2</td>
</tr>
<tr>
<td>Substrate modification, n (%)</td>
<td>11 (58)</td>
<td>13 (100)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

LV indicates left ventricle; LA, left atrium.

Informed consent to the study, which was approved by the institutional Clinical Research and Ethics Committee.

Electrophysiology Study

All patients had anticoagulation for ≥1 month (international normalized ratio, 2 to 3) and transeosophageal evaluation to exclude atrial thrombus before the procedure. Electrophysiological study was performed in the postabsorptive state with sedation with midazolam and morphine. The left atrium (LA) was accessed transseptally, after which a single bolus of 50 IU/kg heparin was administered and repeated only for procedures lasting ≥4 hours. The following catheters were used for mapping and ablation: a steerable quadripolar catheter (5-mm electrode spacing, Xtrem, ELA Medical) positioned within the coronary sinus (CS) with the proximal electrode at 4 to 5 o’clock along the mitral annulus in the left anterior oblique projection, a circumferential mapping catheter (Lasso, Biosense-Webster) introduced after transseptal access and stabilized with the aid of a long sheath (Preface multipurpose, Biosense-Webster) that was continuously perfused with heparinized 5% dextrose, and a 4-mm irrigated-tip ablation catheter (Navi-Star, Biosense-Webster).

Surface ECG and bipolar endocardial electrograms were continuously monitored and stored on a computer-based digital amplifier/recorder system (Bard Electrophysiology). Intracardiac electrograms were filtered from 50 to 500 Hz and measured with online calipers at a sweep speed of 100 mm/s.

Study Protocol

Mapping of Atrial Fibrillation

Before ablation, all patients underwent electroanatomic mapping (CARTO, Biosense-Webster) during AF. The CARTO mapping system has previously been described in detail; it has a sensor position accuracy of 0.8 mm and 5°. The Navi-Star catheter, the 3D geometry of the chamber is reconstructed in real time, and at each point, the system records the 12-lead ECG and bipolar electrograms sampled at 1 kHz and filtered at 30 to 400 Hz, thus allowing the electrophysiological information to be color coded and superimposed on the anatomic map.

We recorded from evenly distributed points using a fill threshold of 20 mm throughout the right atrium (RA), LA, and CS. At each point, 5-second bipolar electrograms, together with the surface ECG, were acquired. Endocardial contact during point acquisition was facilitated by fluoroscopic visualization of catheter motion, the distance to geometry signaled by the catheter icon on the CARTO system, and confirmed in a subset with intracardiac echocardiography by visualizing the catheter-tissue interface (Acunav, Siemens Medical). After completion of the chamber geometry, using a double-blind study design, we performed ablation as described below with the operator blinded to the results of signal processing.

Signal Analysis

Spectral analysis of the recordings obtained by the CARTO system was performed by investigators who were blinded to the baseline characteristics of the patient and the results of ablation. The signals were tapered at their edges to a 0 value by the Hanning window, rectified, and processed with a nonbiased 3- to 15-Hz band-pass filter, thus minimizing the double counting of bipolar double potentials <50 ms. A 4096-point fast Fourier transformation (spectral resolution, 0.24 Hz) was used to obtain the power spectrum of the electrogram at each recording site, and in each spectrum, the frequency with the largest amplitude was assigned to be the DF at that site. To ensure reliability in DF detection, we calculated the regularity index (RI), defined as the ratio of the power at the DF and its adjacent frequencies (≈0.75-Hz band) to the power of the 3- to 15-Hz band. Only points demonstrating RI >0.2 were included in subsequent analysis to control for ambiguity in DF detection related to poor signal-to-noise ratio.

To evaluate the reliability of the 5-second recording interval and the spatiotemporal stability of the power spectrum, the following were performed. In 13 patients, 30-second electrogram recordings were acquired from each of the pulmonary veins (PVs). The DF of each sequential 5-second interval was determined to evaluate the stability of the DF over the entire recording period. In 5 patients, each point over the entire map was acquired for 10 seconds. The DF of each 5-second interval for each point was determined to evaluate the stability of DF throughout the mapped area. For the purposes of this analysis, each point was treated as an individual observation. In 5 additional patients, repeated mapping was performed in each of the PVs and the RA appendage over a 15-minute interval. At each site, the DF of each time point was determined to evaluate the medium-term stability of DF. In 11 patients, recordings were collected from the stable catheter within the CS over 10 equally spaced intervals beginning at the start of mapping and ending with the last point acquired. The DF of each recording was determined to establish the stability of the DF for the duration of the mapping study.

Frequency Mapping and Correlation With Ablation

Power spectral analysis allowed automatic determination of the DF for each point acquired. Using the anatomic geometry, we created 3D color-coded DF maps, displaying low frequencies in red and high frequencies in purple. On DF mapping, sites demonstrating high-frequency activity relative to the surrounding atrial tissue with a decreasing frequency gradient ≥20% to the surrounding points were defined as DF sites. To evaluate potential regional clustering of DF sites, the locations of the various sites were grouped as PV or ostial LA, LA, RA, or CS.

Monitoring the Effect of Ablation

The sequence of ablation was noted and its effect on the AF process was determined using the change in AF cycle length (AFCL) within the CS and the termination and inducibility of AF as previously described.

The AFCL within the CS was determined by averaging the interval of 30 consecutive cycles before and after ablation of each PV using automated CL monitoring software (Bard Electrophysiology). Interelectrogram intervals of <100 ms and continuous electrical activity were counted as a single interval. At each time point, the automated annotation was manually verified and corrected with online calipers at a paper speed of 100 mm/s by a single investigator. In 10 randomly selected patients, AFCL was determined by 2 independent investigators to control for intra and interobserver variation.

If AF terminated during ablation, the AFCL was determined before termination. To avoid transitional changes at this point, the AFCL was determined 10 cycles before termination. The sites of AF termination were noted. Termination of AF was defined as previously described as direct transition to sinus rhythm or to flutter.
The relationship between DF sites and the site at which ablation led to AF termination was assessed by 2 investigators; differences were resolved by consensus.

In patients in whom AF terminated during ablation, the inducibility of AF was determined using a previously described protocol after isolation of the PV or after completion of linear ablation.15 In brief, burst atrial pacing (5 seconds, 20 mA) from the CS and RA and LA appendages was sequentially performed beginning at a CL of 250 ms and reducing by 10-ms intervals until refractoriness. AF was considered inducible if it persisted for ≥10 minutes. If AF terminated within 10 minutes, induction was repeated at least 3 times from each site.

For the purposes of this study, AF was defined by the beat-to-beat variability in CL and morphology, whereas atrial flutter was defined as a rapid regular atrial rhythm with stable CL, morphology, and variability in CL and morphology, whereas atrial flutter was defined as a rapid regular atrial rhythm with stable CL, morphology, and activation sequence.

**Radiofrequency Ablation of AF**

The techniques used for ablation in this cohort of patients include PV isolation and ablation of the cavotricuspid isthmus as the first step. In patients with persistent or inducible AF after the above strategy, additional substrate modification was performed. If focal activity was observed after reversion to sinus rhythm, it was mapped and focal ablation was performed.

**PV Electrical Isolation**

Ablation of the PVs was performed to electrically isolate all veins without any attempt to identify potentially arrhythmogenic PVs at the time of the procedure. This was performed individually or as a pair when the ostia were coalescent. Ablation began ~1 cm from the ostium of both right PVs and for the posterior and superior aspects of the left PVs. However, when ablation was required at the anterior portions of the left PVs, energy was delivered by inferring slightly into the first part of the vein to achieve catheter stability. The procedural end point was complete elimination or dissociation of PV potentials. Radiofrequency energy was delivered with power up to 30 W (inside the PV) to 35 W (at the ostia) using irrigation rates of 5 to 20 mL/min (0.9% saline via Cool Flow, Biosense-Webster) to achieve the desired power delivery. Temperature was limited to 50°C.

**Substrate Modification**

Linear ablation of the cavotricuspid isthmus was performed in all patients with an end point of bidirectional conduction block. Additional substrate modification was performed by linear ablation to join anatomic structures (between the PVs or joining the PV to the mitral annulus) as previously described.12-15 The end point of these procedures was the demonstration of complete linear block by continuous online double potentials with an activation detour that was confirmed, when possible, by differential pacing techniques. Radiofrequency energy was delivered with power limited to 30 to 40 W using irrigation rates of 5 to 60 mL/min to achieve the desired power delivery. Temperature was limited to 50°C.

**Statistical Analysis**

Continuous variables are reported as mean±SD and assessed for normality using the Shapiro-Wilk test. Normally distributed data were compared through the use of paired or unpaired Student t test. Data not normally distributed were compared by use of the Wilcoxon signed-rank or rank-sum test for paired and unpaired data, respectively. Sequential data measurements were analyzed by repeated-measures ANOVA, followed by Scheffe’s comparison. Categorical variables are reported as number and percentage and compared through the use of Fisher’s exact test. Intraobserver variability and interobserver variability of AFCL determination were assessed with the single measure intraclass correlation. The χ coefficient for concordance was used to determine the relationship between DF sites and an increase in AFCL (≥5 ms) with ablation. Concordance was considered strong if χ>0.6 as previously described.16 Statistical significance was established at P<0.05.

**Results**

**Patient Characteristics**

Patients had either paroxysmal (n=19) or permanent AF of long duration (18.4±16.6 months; range, 6 to 60 months; n=13) and were undergoing AF ablation, having failed 3.5±1.5 antiarrhythmics (including amiodarone in 20 patients). Six patients with paroxysmal AF had spontaneous arrhythmia for 5.4±4.1 hours (range, 1.5 to 12 hours) before the procedure; in the remaining 13, it was induced by burst pacing and was sustained (at least 10 to 15 minutes) before mapping. However, although AF persisted for 2.3±1.3 hours in 11 of these 13 patients, there was recurrent interruption of arrhythmia during mapping in 2 patients. Those with permanent AF had significantly larger LA diameters and demonstrated a trend to more structural heart disease and decreased left ventricular function.

**Spatiotemporal Stability of Frequency During AF**

We acquired 126±13 points per patient: 56±8 points per patient in the LA and PVs, 52±8 points per patient in the RA, and 18±4 points per patient in the CS; 79% fulfilled the predefined criteria of regularity for inclusion in the DF maps.

The temporal stability of spectral analysis was determined by evaluating sequential segments of 5 seconds as detailed in Methods. In the PVs, sequential intervals over 30 seconds demonstrated temporal stability of the DF in each PV with a range of DF as follows: left superior PV, from 6.5±1.0 to 6.9±0.9 Hz (P=0.7); left inferior PV, from 6.1±1.4 to 6.6±1.2 Hz (P=0.5); right superior PV, from 5.8±0.8 to 6.5±1.1 Hz (P=0.3); and right inferior PV (RIPV), from 6.0±1.0 to 6.6±1.2 Hz (P=0.4).

In 596 points, two 5-second sequential intervals acquired over 10 seconds throughout both atria and CS revealed spatiotemporal stability of DF throughout the mapped field with no significant variability between the DF determined during the first (6.2±1.8 Hz) and second (6.3±1.9 Hz) intervals or the entire 10-second recording interval (6.2±1.7 Hz; P=0.4).

Repeated sequential analysis from the RA appendage and PVs demonstrated temporal and spatial stability of DF over a 15-minute interval with a range of DF at each site as follows: left superior PV, from 5.9±0.8 to 6.4±0.8 Hz (P=0.5); left inferior PV, from 5.8±0.3 to 6.2±0.8 Hz (P=0.5); right superior PV, from 5.2±0.4 to 5.8±1.0 Hz (P=0.2); RIPV, from 5.4±0.3 to 6.0±0.7 Hz (P=0.2); and RA appendage, from 5.1±1.0 to 6.4±1.9 Hz (P=0.2).

Finally, sequential 5-second intervals within the CS for the entire duration of the study protocol demonstrated temporal stability of DF for 35 to 48 minutes (P=0.9); the mean DF was 5.3±0.6 Hz with a difference between the maximum and minimum recorded DF of 1.1±0.5 Hz.

**Frequency Mapping and Spatial Distribution of DFs**

Both groups of patients demonstrated a regional maximal frequency gradient from LA to RA to CS (P=0.0005; Figure 1). However, compared with patients with paroxysmal AF, patients with permanent AF globally demonstrated a higher average frequency of activity (P=0.0006), which persisted in each chamber: LA, 6.4±0.6 versus 5.5±0.6 Hz (P<0.01);
RA, 6.2±0.8 versus 5.3±0.7 Hz (P<0.01); and CS, 6.3±0.9 versus 5.4±0.8 Hz (P<0.01).

Although the DF value of each point allowed assessment of its role as a DF site (as defined), this could be further appreciated by the creation of a 3D DF map. Although most DF sites involved a single point, the DF site was spread over 2 to 3 adjacent points in a few sites (particularly in permanent AF). Figure 2 shows 4 bipolar signals with their respective spectra acquired from endocardial sites in the LA and RA in a patient with paroxysmal AF of spontaneous onset. On the left are typical AF recordings with variable amplitudes and interbeat intervals, which, particularly in the first recording, precluded CL analysis. However, the spectra on the right show relatively narrow bands in the 3- to 15-Hz range with distinct peaks of DFs. In this patient, 120 electrograms were collected, and the corresponding DFs were superimposed on the atrial geometry to generate the DF map (Figure 3A). Figure 3A presents 2 views showing activity at mean DF of 4.8±0.9 Hz. Although frequency in most of the atria and CS was relatively slow (<5 Hz), the posterior wall of the LA was activated at a faster rate, with notable DF sites at each of the PVs. In this patient, the arrhythmia terminated during ablation at the DF site near the RIPV.

Figure 3B shows a DF map from a patient with permanent AF. Compared with the patient with paroxysmal AF, not only does this patient have a higher frequency at the maximal DF site (13.7 Hz), but also both atria demonstrate higher global frequency of activity (6.7±1.1 Hz; n=119 points; P<0.0001 versus patient in Figure 3A). Compared with the electrograms from the DF sites in paroxysmal AF (Figure 2), the activity at the DF site of this patient is more fractionated and is reflected by the secondary band in the corresponding power spectrum.

We evaluated the spatial distribution of DF sites in each group in terms of the PV/ostial LA and the rest of the LA, RA, and CS (Figure 4 and 5). In paroxysmal AF, the PV/ostial LA region was most likely to harbor a DF (42%); that probability decreases toward the rest of the atria and CS. In contrast, although permanent AF patients show a similar incidence of DF sites in the RA and CS, the number tends to be reduced in the PV/ostial LA region, with only 26% of DF sites localized to that region. Indeed, permanent AF patients seem more likely to have DF sites localized to other non-PV regions of the LA compared with paroxysmal AF patients (Figure 4). In addition, the site with the maximum DFs during mapping was localized to the PV more often in patients with paroxysmal than in those with permanent AF (one third of patients versus 0, respectively; P=0.02).

**Change in AFCL With Ablation**

Ablation of the PVs was associated with a significant increase in the AFCL determined within the CS from 174±27 (range, 130 to 259 ms; n=88) to 184±35 ms (range, 132 to 314 ms; P<0.0001). However, ablation at a PV harboring a DF site resulted in AFCL prolongation, increasing from 180±30 (range, 130 to 259 ms; n=44) to 198±40 ms (range, 132 to 314 ms, P<0.0001; Figure 3A), whereas ablation at a PV without a DF site did not change the AFCL, which was 169±22 ms (range, 135 to 219 ms; n=44) before and
170±22 ms (range, 137 to 224 ms, P=0.4; Figure 3B) after ablation. Ablation at PVs harboring a DF site resulted in an increase in AFCL (≥5 ms) within the CS in 89% and was observed in patients with paroxysmal and permanent AF, with the mean increase in AFCL being 18±21 ms (range, 0 to 118 ms; n=44) compared with 0.9±3.9 ms (range, −10 to 7 ms; n=44; P<0.0001) after ablation at PVs without a DF site. The increase in AFCL with PV ablation demonstrated a strong concordance with ablation at a DF site, with a χ of 0.77.

The intraobserver and interobserver variability assessment for AFCL determination showed good reproducibility, with interclass correlation coefficients of 0.97 and 0.98, respectively.

**Termination of Arrhythmia With Ablation**

Arrhythmia termination occurred during ablation in 17 of 19 patients with paroxysmal and 0 of the 13 with permanent AF (P<0.0001). However, 2 of these 17 patients had frequent cessations of AF during mapping, with cessation in 1 at the start of the first PV ablation and the other immediately after isolation of the first PV (terminating 3 and 4 times, respectively, before ablation). When these 2 patients are excluded, 15 of the 17 paroxysmal AF patients (88%) with sustained...
arrhythmia during mapping terminated with ablation. As classified in Table 2, in 13 of these 15 patients (87%), arrhythmia termination was associated with ablation at a DF site: 11 localized to a PV and 2 to the atria. In the latter 2 patients, the high-frequency activity maintaining AF was localized to the LA roof and the fossa ovalis (Figure 6A).

In 7 patients (54%), the DF site terminating arrhythmia was the initial DF site encountered during ablation. In patients with AF termination, 1.9±1.0 DF sites per patient (38±21% of DF sites) were ablated before the termination of arrhythmia, with a total of 2.3±1.0 DF sites per patient being ablated during the procedure. However, in patients with paroxysmal AF, there was no significant difference in the frequency of the DF site that resulted in AF termination compared with those at which AF persisted despite ablation (8.9±1.9 versus 8.8±1.2 Hz; \(P=0.8\)).

In 2 patients with paroxysmal AF, arrhythmia persisted despite PV isolation and substrate modification. In both patients, blinded analysis revealed that DF sites were also located outside the ablated zones (1 in each patient; Figure 6B). In all patients with permanent AF, when AF persisted despite extensive substrate modification in some cases, at least 1 DF site had not been ablated (mean, 2.8±1.4 DF sites per patient not ablated). The frequency of ablated DF sites in patients with persistent AF was 9.0±2.2 Hz and was not significantly different from those that terminated AF in patients with paroxysmal AF (8.9±1.9 Hz; \(P=0.8\)).

**TABLE 2. Termination of AF During Ablation and Its Relationship to DF Sites**

<table>
<thead>
<tr>
<th>Ablation Site</th>
<th>n</th>
<th>DF Site</th>
<th>Independent of DF</th>
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<tr>
<td>PVs</td>
<td></td>
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<tr>
<td>Paroxysmal</td>
<td>14</td>
<td>11</td>
<td>3*</td>
</tr>
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</tr>
<tr>
<td>Permanent</td>
<td>0</td>
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</tbody>
</table>

*Two had frequent cessation of arrhythmia during mapping.

To further investigate the nature of the differences in ablation results between patients with paroxysmal and permanent AF, we determined the relation that existed between the DF sites and the ablation sites, as presented in Table 2. There were 5.2±1.8 (range, 1 to 10) separate DF sites per patient, with no significant difference in the number of DF sites between patients with permanent or paroxysmal AF (5.3±1.9 versus 5.1±1.9 sites per patient, respectively; \(P=0.9\)). In addition, there was no significant difference in the number (1.9±1.0 versus 2.1±1.4 DF sites per patient, respectively; \(P=0.7\)) or percentage (38±21% versus 40±24% of DF sites in each patient, respectively; \(P=0.8\)) of DF sites ablated in patients with and without AF termination during ablation. However, the duration of radiofrequency energy delivered to achieve termination was significantly shorter than that delivered before electrical cardioversion in those with persisting AF: 34.8±24.0 versus 73.5±22.9 minutes, respectively (\(P=0.0002\)). Using a double-blinded study design, ablation performed without knowledge of the DF sites resulted in significantly shorter radiofrequency duration per DF site ablated in patients with AF termination compared with those with ongoing AF (21.9±18.6 versus 42.4±26.4 minutes; \(P=0.008\)), implicating a potentially altered distribution of DF sites in those without AF termination. In addition, after termination of AF during ablation, rigorous burst pacing could not induce sustained AF in 9 of 13 patients (69%) in whom AF terminated at a DF site.

**Discussion**

This study presents new information regarding the identification and role of localized sites of high-frequency activity in the maintenance of AF in humans. First, it demonstrates the feasibility of performing spectral analysis and endocardial DF mapping in humans to recognize relatively small DF sites with spatiotemporal stability in a 3D representation with a hierarchical gradient of activity. Second, it identifies that, in patients with paroxysmal AF, the DF sources of activity are often localized to the PVs. In contrast, patients with permanent AF demonstrate DF sites that are more often localized to the atria, including RA sites. Finally, it demonstrates the importance of identifying these DF sites in humans with AF.
Ablation at these DF sites resulted in a significant slowing of the fibrillatory process and termination of sustained AF in 87% of patients with paroxysmal AF, confirming the role of localized sites of high frequency in the maintenance of AF in humans.

Localized Sources of Activity in AF

The notion that a localized source of activity could maintain AF was put forth by early investigators such as Lewis\textsuperscript{17} and Scherf.\textsuperscript{18} More recently, in an isolated canine atrial preparation, Schuessler et al\textsuperscript{3} observed that, with increasing concentrations of acetylcholine, activation patterns characterized by multiple reentrant circuits converted to a single, relatively stable, high-frequency reentrant circuit that resulted in fibrillatory conduction. Using a sterile pericarditis model, Kumagai et al\textsuperscript{19} identified dominant unstable reentrant circuits of very short CL at the septum maintaining AF that could be successfully ablated focally. Morillo et al\textsuperscript{20} targeted ablation to sites of short CL activity in the posterior LA and observed the termination of arrhythmia in a canine model of AF. In humans, we have reported that in some patients sustained focal activity initiated and maintained AF and could be eliminated by discrete ablation at the PV, CS, or superior vena cava.\textsuperscript{21}

Studies performing mapping during AF have recognized the presence of temporally and spatially periodic activity\textsuperscript{6,7,22,23} emanating from the PV region with regularity,\textsuperscript{5} suggesting that these structures may have a role in maintaining AF through localized short CL reentry and/or focal high-frequency activity.\textsuperscript{24,25} A recent study using a morphologically accurate computer model of the atria has demonstrated the PV region to be a preferential site of anchoring a rotor.\textsuperscript{26} In humans, similar observations of paroxysmal short CL activity have been observed in the PVs during AF ablation.\textsuperscript{27–29} This has been associated with a progressive slowing of the AF process, culminating in the termination of AF in 75% of patients with paroxysmal AF during PV isolation.\textsuperscript{11}

The present findings extend these observations by demonstrating that ablation at PVs harboring DF sites resulted in the slowing of the AF process, termination of AF, and noninducibility of AF in a significant percentage, thus implicating this activity in the substrate maintaining AF. Importantly, multiple DF sites were observed in a given individual, and although ablation at each site resulted in slowing of the AF process, termination was observed at not just the maximal DF site. This observation could be evidence for the role for multiple sources of activity in the maintenance of AF or proof that while there was spatial stability of DF sites, the sequential mapping technique used may have been sufficiently prolonged to allow 1 of these predisposed sites to become the maximal DF site. The latter has recently been observed in simulations of AF using a morphologically accurate model of the atria.\textsuperscript{26} The ability of spectral analysis and frequency mapping to identify sources of activity maintaining AF raises the possibility of its use to identify the substrate for AF during ablation procedures.

Frequency Mapping to Identify Sources Maintaining AF

Mapping during AF is notoriously difficult to interpret. The incessantly changing activation patterns within the atria result in complex, fractionated signals and potentials separated by short intervals on intracardiac electrograms that hinder the reliable interpretation and identification of regions of very short CL activity.\textsuperscript{30,31} Herein lies the utility of frequency mapping, which allows localization of sites of rapid and periodic activity that are likely to be closely linked to sources that maintain AF.\textsuperscript{5,6,8,32} In addition, the rotor frequency identified by optical mapping has been found to correlate well with the DF determined by signal analysis,\textsuperscript{33} suggesting that DF mapping is capable of identifying these “rotors” maintaining AF. In the present study, ablation of DF sites

Figure 6. A, DF map of patient with arrhythmia termination at non-PV site. Tilted left anterior oblique projection demonstrates DF site located at fossa ovalis. Local ablation terminated tachycardia within seconds of start of ablation. B, DF map of patient with paroxysmal AF persistent after PV isolation and substrate modification. Posterior-anterior projection of DF map is presented. In this patient, AF persisted after PV isolation, roofline, and mitral isthmus ablation. No ablation had been performed within CS, the only DF site in entire map.

Ablation at these DF sites resulted in a significant slowing of the fibrillatory process and termination of sustained AF in 87% of patients with paroxysmal AF, confirming the role of localized sites of high frequency in the maintenance of AF in humans.
coincided with slowing and termination of AF in 87% of patients in whom arrhythmia terminated during ablation. Whether such sites are reentrant or focal in humans remains to be determined.

In a recent report by Nademanee et al., ablation performed at sites of complex fractionated electrograms was observed to result in the termination of AF. Importantly, short CL activity (<120 ms) was included in their definition of fractionated electrograms. However, they found fractionation throughout both atria (in 7 of 9 areas) and required extensive ablation. Although we did not evaluate the relationship with electrogram fractionation, it is possible that a region of high-frequency activity may manifest also as complex fractionated or continuous activity (see Figures 2 and 3B).

In persistent AF, several investigators have suggested a distinct difference and loss of spatiotemporal organization. In our study, patients with persistent arrhythmia had a similar number and percentage of DF sites that had been ablated, although doing so required a significantly greater duration of radiofrequency application. Failure of termination in this cohort may indicate that (1) ablation did not include critical DF sites maintaining AF (because of the different distribution of these sites), (2) a frequency gradient was not recognized because of the ability of the remodeled atria with short refractoriness to be activated at similar frequency as the source, or (3) the mechanisms underlying the maintenance of AF in these patients are different. A previous intraoperative mapping study in patients with permanent AF and organic heart disease reported the presence of rapid repetitive activity originating from the corners of the electrode plaque placed on the posterior LA that were suggestive of a PV origin. In addition, Sahadevan et al. have observed regular short CL activity, predominantly from the LA, in patients with chronic AF and suggested that the posterior LA may be responsible for maintaining chronic AF. However, Nitta et al. have observed the existence of multiple LA and RA focal activations in patients with permanent AF and mitral valve disease that may explain the maintenance of AF despite ablation in these patients.

Clinical Implications
Catheter ablation strategies for the cure of AF have targeted isolation of the PVs, whereas additional substrate modification is required in 20% to 40% of patients with paroxysmal AF and most with persistent or permanent AF. Linear ablation to modify the atrial substrate has provided variable outcomes but is challenging, is associated with an increased procedural risk, and may be proarrhythmic. A more localized and targeted approach to substrate modification has inherent advantages. The present study suggests that spectral analysis and frequency mapping may constitute a novel modality of substrate mapping for the ablation of AF.

Study Limitations
Mapping in this study was performed sequentially and therefore assumed temporal and spatial stability of the activity. Although we demonstrated short- and medium-term temporal stability, evaluation of longer-term stability was not feasible because of the duration of the study protocol. Nevertheless, the longer-duration temporal and spatial stability of these sources has been demonstrated in previous experimental studies and is highly likely to exist in humans, given the observations of AF termination with ablation at DF sites in the current cohort.

The time frame analyzed was selected on the basis of previous experimental data and was demonstrated to be reproducible in a subset in this cohort. However, it is feasible that infrequent bursts of activity may not have been detected.

Finally, this initial study validating the use of spectral analysis and frequency mapping in humans necessarily required a double-blind study design. These results now need to be confirmed in a prospective study using real-time DF analysis.

Conclusions
Frequency mapping identifies localized sites of high-frequency activity during AF in humans with different distributions in paroxysmal and permanent AF. Ablation at these sites results in a slowing and termination of paroxysmal AF, indicating their role in the maintenance of AF. Spectral analysis and frequency mapping may constitute a novel modality of substrate mapping for the ablation of AF.

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