ORIGINAL ARTICLES

WILEY

Location and coupling interval of an ectopic excitation determine the initiation of atrial fibrillation from the pulmonary veins

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Abstract

Introduction: Ectopic beats originating from the pulmonary vein (PV) trigger atrial fibrillation (AF). The purpose of this study was to clarify the electrophysiological determinant of AF initiation from the PVs.

Methods: Pacing studies were performed with a single extra stimulus mimicking an ectopic beat in the left superior PVs (LSPVs) in 62 patients undergoing AF ablation. Inducibility of AF, effective refractory period (ERP), and conduction properties within the PVs were analyzed.

Results: A single extra stimulus in LSPV induced AF in 20 patients (32% of all patients) at the mean coupling interval (CI) of 172 ms. A CI-dependent anisotropic conduction at the AF onset was visualized in a three-dimensional mapping. Onset of AF was site-specific with reproducibility in each individual. Mean ERP in LSPV in the AF-inducible group was shorter than that in the AF-noninducible group (182 ± 55 vs. 254 ± 51 ms, $p < .0001$). LSPV ERP dispersion was greater in the AF-inducible group than in the AF-noninducible group (45 ± 28 vs. 27 ± 19 ms, $p < .01$). Circumferential intra-PV conduction time (IPVCT) exhibited decremental properties in response to shortening of CI and the prolongation of IPVCT in the AF-inducible site was greater than that in the AF-noninducible site ($p < .05$) in each individual.

Conclusions: Location and CI of an ectopic excitation ultimately determine the initiation of AF from the PVs. ERP dispersion and circumferential conduction delay may lead to anisotropic conduction and reentry within the PVs that initiate AF.

KEYWORDS
atrial fibrillation, catheter ablation, decremental properties, effective refractory period, site specificity

1 | INTRODUCTION

It has been demonstrated that atrial fibrillation (AF) is triggered by ectopic beats in the pulmonary veins (PV).[1,2] Since then, a number of studies regarding electrophysiological properties in the PVs have been reported. It has been demonstrated that the effective refractory period (ERP) of the PVs in patients with AF was shorter than that in control patients without AF, whereas the ERP of the left atrium (LA) was not significantly different.[3] The ERP heterogeneity and anisotropic conduction within the PV have been reported as well, which may be the crucial electrophysiological properties in the onset of AF.[4] In an observation of spontaneous AF firing within the PVs using a basket catheter, the coupling interval (CI) of ectopic beats initiating AF was shorter than that of ectopic beats not initiating AF.[5] However, little is known about the ultimate determinant factors of AF initiation and no previous studies clearly could explain the reasons why all the ectopic beats from the PVs could not trigger AF. The purpose of this study was to clarify the electrophysiological determinant factors of AF initiation from the PVs.

2 | METHODS

2.1 | Study population

This study enrolled 62 patients with drug-refractory AF referred to our institution for AF ablation; 25 patients from Kyushu University Hospital between October 2013 and December 2016, and 37 patients from Japanese Red Cross Fukuoka Hospital between September 2019 and March 2020. We enrolled only first-session cases and multiple session cases were excluded. The definition of paroxysmal AF and persistent AF was followed by the American College of Cardiology/American Heart Association/Heart Rhythm Society guidelines.[6] In short, paroxysmal AF was defined as episodes lasting <7 days and self-terminated; persistent AF was defined as episodes lasting >7 days. Antiarrhythmic drugs were discontinued at five half-lives before ablation. No patients were treated with amiodarone. The presence of LA thrombi was excluded by contrast-enhanced computed tomography. When a contrast defect in the left atrial appendage was suspected, we excluded the presence of LA thrombi by transesophageal echocardiography. This study was in compliance with the principles outlined in the Declaration of Helsinki and was approved by the institutional review board for ethics at our institution, Kyushu University Hospital (approval number 29-44). Informed consent was obtained in the form of opt-out on the website (https://www.cardiol.med.kyushu-u.ac.jp/research/clinical-research/).

2.2 | Electrophysiological study

All patients underwent an electrophysiological study in the fasting state under conscious sedation. A 20-pole catheter was inserted
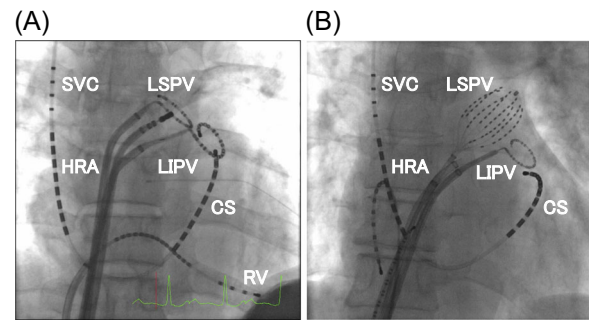

FIGURE 1 Catheter position during pulmonary vein pacing. (A) Twenty-pole circular mapping catheters are positioned in LSPV and LIPV. (B) Sixty-four-pole basket catheter is positioned in LSPV and 20-pole circular mapping catheter is positioned in LIPV. CS, coronary sinus; HRA, high right atrium; LIPV, left inferior pulmonary vein; LSPV, left superior pulmonary vein; RV, right ventricle; SVC, superior vena cava

through the right jugular vein (BeeAT® Japan Lifeline). The proximal portion of the catheter was positioned along the superior vena cava and crista terminalis, and the distal portion was positioned in the coronary sinus (CS) for pacing and internal cardioversion.

Following a *trans*-septal puncture under guidance with an intracardiac echocardiography catheter (5.5–10 MHz, 8Fr, AcuNav™, Biosense Webster), two or three long sheaths (SL1®, AF Division, St. Jude Medical) were introduced into the LA via the same *trans*-septal puncture site. After a left atriography was performed, 20-pole circular mapping catheters (1–5–1 mm interelectrode spacing, 20 mm in diameter, and/or 1–3.5–1 mm interelectrode spacing, 15 mm in diameter) and a 3.5 mm open-irrigated-tip ablation catheter (Navistar® Thermocool®, Biosense Webster or Thermocool Smarttouch®, Biosense Webster, or TactiCath™, Abbott) were positioned in the PVs for PV mapping (Figure 1A). In four patients, PV mapping was also performed with a 64-pole basket catheter (Constellation®, Boston Scientific) (Figure 1B). The electrophysiological studies were performed under support of an electroanatomical mapping system with the CARTO® system (Biosense Webster) or Ensite Velocity™ system (St. Jude Medical).

2.3 | Stimulation protocol

PV pacing was performed from a circular mapping catheter or from the proximal (bipoles 3–4 or 5–6) electrode pair of a basket catheter located in the left superior PV (LSPV). A cardiac stimulator (SEC-5104) was used to deliver electrical impulses of 1 ms duration at an amplitude of 10 V, the negative pole being connected to the distal electrode of the pacing catheter. Electrocardiographic leads and intracardiac electrograms filtered at 30–150 Hz were recorded simultaneously with a polygraph (Cardio LAB IT, GE Healthcare).

A single extra stimulus coupled at 400 ms was decremented automatically in steps of 10 ms to the ERP without a basic drive pacing, mimicking a spontaneous firing from the PVs and in

FIGURE 2 Details of pacing-induced AF.
(A) Distribution of atrial fibrillation
(AF)-inducible sites. Three-dimensional
mapping shows left superior pulmonary vein.
Each yellow dot indicates an AF-inducible
case. (B) Histogram of coupling interval of
pacing stimuli that induced AF
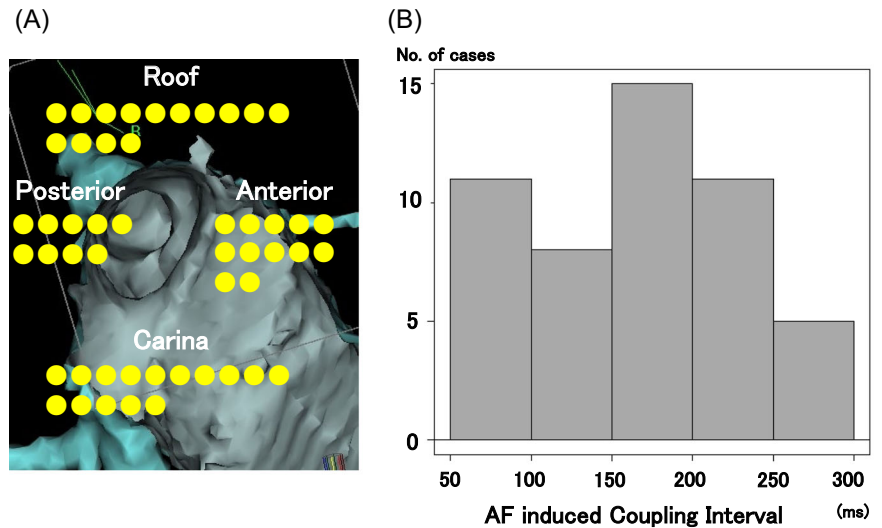
consideration of the influence of a basic drive pacing on the ERP.[2,7] Pacing stimuli were delivered at multiple sites (anterior, roof, posterior, and carina). The sinus cycle length in each patient was set between 600 and 700 ms under administration of intravenous isoproterenol (ISP). Cases with or without AF induction following a single extra stimuli were defined as AF-inducible and -noninducible group, respectively. Reproducibility of AF induction with the same CI was confirmed after AF termination.

The following variables were measured: (1) ERP at each pacing site in the LSPV. The ERP was defined as the longest CI at which a single extra stimulus failed to capture local myocardial sleeve within the PV. (2) Circumferential intra-PV conduction time (IPVCT) was measured from the pacing artifact to the end of the local electrical excitation within the plane of the circular mapping catheter at each CI. The time phase at which electrical potential returned to the baseline was compared among the electrode pairs and the latest time phase was considered as the end of the local electrical excitation within the assessed circular plane. The electrode pair where a pacing stimulus was delivered was considered as the initiation site of the electrical excitation. (3) PV-LA conduction time was measured from the pacing artifact to the end of the local electrical excitation at the electrode pair positioned in the CS distal at each CI.

In AF-inducible cases, circumferential IPVCT and PV-LA conduction time in AF-inducible pacing sites were compared with those in AF-noninducible pacing sites. Sequential change of intra-PV and PV-LA conduction were assessed from +50 ms to the CI of AF onset in the AF-inducible sites, whereas from +60 to +10 ms of ERP in the AF-noninducible sites. Mean value was adopted in cases that multiple AF-inducible sites and/or AF-noninducible sites existed.

2.4 | Statistical analysis

The data are expressed as mean ± SD for continuous variables, and counts and percentages for categorical variables. A comparison of categorical variables between pairs of groups was carried out using the

TABLE 1 Patient characteristics

Characteristics	Total (n = 62)	AF inducible (n = 20)	AF noninducible (n = 42)	p
Age (y)	62.3 ± 11.1	67.6 ± 11.5	65.0 ± 12.3	.44
Male	43 (69.3)	14 (70.0)	29 (69.0)	1.0
Hypertension	40 (64.5)	13 (65.0)	27 (64.3)	1.0
Structural heart disease	6 (9.7)	1 (5.0)	5 (11.9)	.65
CHADS2 score	1.4 ± 1.2	1.6 ± 1.4	1.2 ± 1.1	.27
LAD (mm)	39.6 ± 6.0	41.4 ± 7.2	38.7 ± 5.2	.1
LAVI (ml/m^2)	38.9 ± 13.0	40.4 ± 13.8	38.2 ± 12.7	.56
LVEF (%)	64.6 ± 7.3	66.1 ± 7.8	63.9 ± 7.1	.27
Paroxysmal AF	40 (64.5)	12 (60.0)	28 (66.7)	.78
ISP (µg)	1.6 ± 1.1	1.5 ± 1.1	1.7 ± 1.1	.64

Abbreviations: ISP, isoproterenol; LAD, left atrial diameter; LAVI, left atrial volume index; LVEF, left ventricular ejection fraction.

χ^2 test or Fisher's exact test. A comparison of continuous variables between pairs of groups was carried out using Student t-test. We performed a two-way analysis of variance for a comparison of the effect of multiple levels of two factors that had multiple observations at each level. All tests were two-sided and $p < .05$ were considered significant. Analyses were conducted using software program EZR.

3 | RESULTS

3.1 | Patient characteristics

A total of 62 patients were enrolled in this study: 43 men (69%), 19 women (31%), and age 66 ± 12 years old. There were 40 paroxysmal AF patients (64.5%) and 22 persistent AF patients (35.5%). Six patients had

structural heart diseases: hypertrophic cardiomyopathy in two patients, old myocardial infarction in two patients, hypertensive heart disease in one patient, and sick sinus syndrome in one patient. A single extra stimulus in LSPV induced AF in 50 sites in 20 patients. LSPV carina was the most prevalent AF-inducible site, followed by the roof (Figure 2A). The mean CI at the onset of AF was 172 ± 69 ms (Figure 2B).

We divided the patients into two groups: AF-inducible group (n = 20, 32.2%) and AF-noninducible group (n = 42, 67.8%). Table 1 summarizes the baseline characteristics of these two groups. There was no significant difference in age, sex, or comorbidities such as hypertension and structural heart disease between the two groups. AF type (paroxysmal AF or persistent AF), CHADS$_2$ score, left atrial diameter, left atrial volume index, left ventricular ejection fraction, and administered amount of ISP were also comparable between the two groups.

3.2 | Inducibility of AF and PV ERP

Mean number of pacing sites in the same case was 4.61. We compared LSPV ERP between the two groups. Mean LSPV ERP in the AF-inducible group was significantly shorter than that in the AF-noninducible group (182 ± 55 vs. 254 ± 51 ms, p < .0001; Figure 3A). In addition, we compared ERP dispersion of LSPVs in each case between the two groups. Mean LSPV ERP dispersion in the AF-inducible group was significantly greater than that in the AF-noninducible group (45 ± 28 vs. 27 ± 19 ms, p < .01; Figure 3B). In response to the shortening of the CI, intra-PV conduction gradually lost the centrifugal activation pattern and progressively elicited anisotropic conduction within the PVs, leading to AF induction

(Figure 4 and Supplementary Movies). We also focused on location specificity. The onset of AF depended on pacing sites of the PVs. Some pacing sites were AF-inducible reproducibly, but other pacing sites, even in adjacent areas, resulted in bare premature atrial contractions (Figure 5). Site-specific reproducibility in each individual was 80% among 20 cases studied. Reproducibility was confirmed 1.46 times. Although most of the induced AF terminated spontaneously within 1–2 min, electrical cardioversion was required in four cases (20%). Twice electrical cardioversion was performed in two cases and once in the rest two cases.

To ensure the aforementioned results of LSPV in another PV, we performed pacing studies in the right superior PV (RSPV) in a separate series of 31 patients. A single extra stimulus in RSPV induced AF in two patients (6.5%), which was significantly lower than that in LSPV (p < .01). In these two AF cases, the AF induction was specific to pacing sites as in LSPV. Reproducibility of AF induction was also confirmed. Mean RSPV ERP was shorter in the inducible two cases compared to the noninducible group (N = 29) (194 vs. 251 ± 48 ms). Mean RSPV ERP dispersion was greater in the inducible two cases compared with the noninducible group (69 vs. 26 ± 13 ms), as observed in LSPV.

3.3 | Quantitative analysis of circumferential intra-PV and PV-LA conduction time

In the AF-inducible cases (n = 20), 14 cases had both AF-inducible sites and AF-noninducible sites studied in LSPVs. Circumferential IPVCT and PV-LA conduction time were evaluated both in the AF-inducible and the -noninducible sites in the same individuals. In the
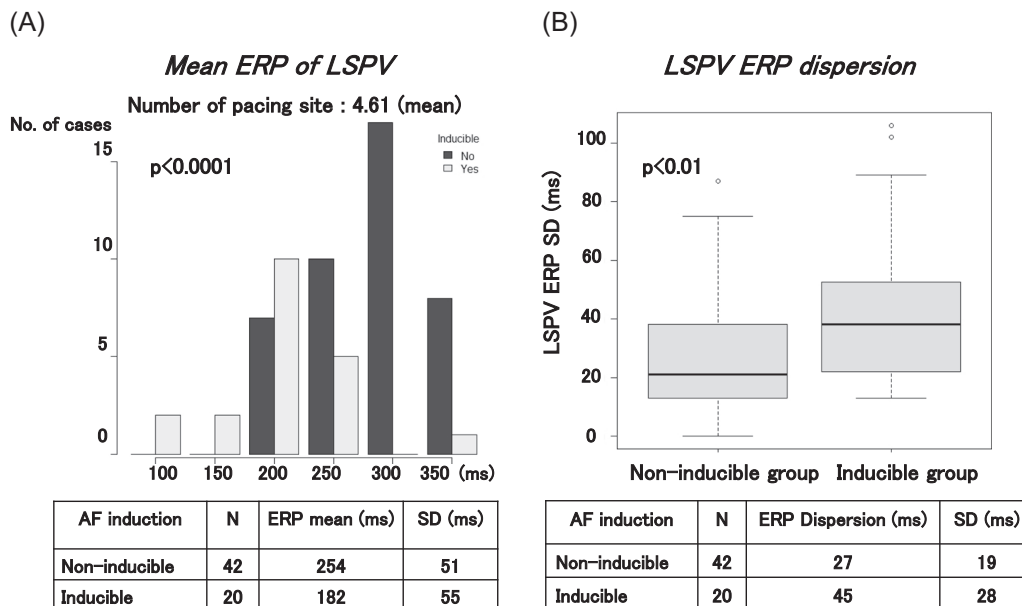

(A)

Mean ERP of LSPV
Number of pacing site : 4.61 (mean)

AF induction	N	ERP mean (ms)	SD (ms)
Non-inducible	42	254	51
Inducible	20	182	55

(B)

LSPV ERP dispersion

AF induction	N	ERP Dispersion (ms)	SD (ms)
Non-inducible	42	27	19
Inducible	20	45	28

FIGURE 3 Difference of mean left superior pulmonary vein (LSPV) effective refractory period (ERP) and LSPV ERP dispersion between atrial fibrillation (AF)-inducible and -noninducible groups. (A) Histogram of LSPV ERP. The black bar indicates the AF-noninducible group and the gray bar indicates the AF-inducible group. (B) Box-and-whisker plot of LSPV ERP dispersion
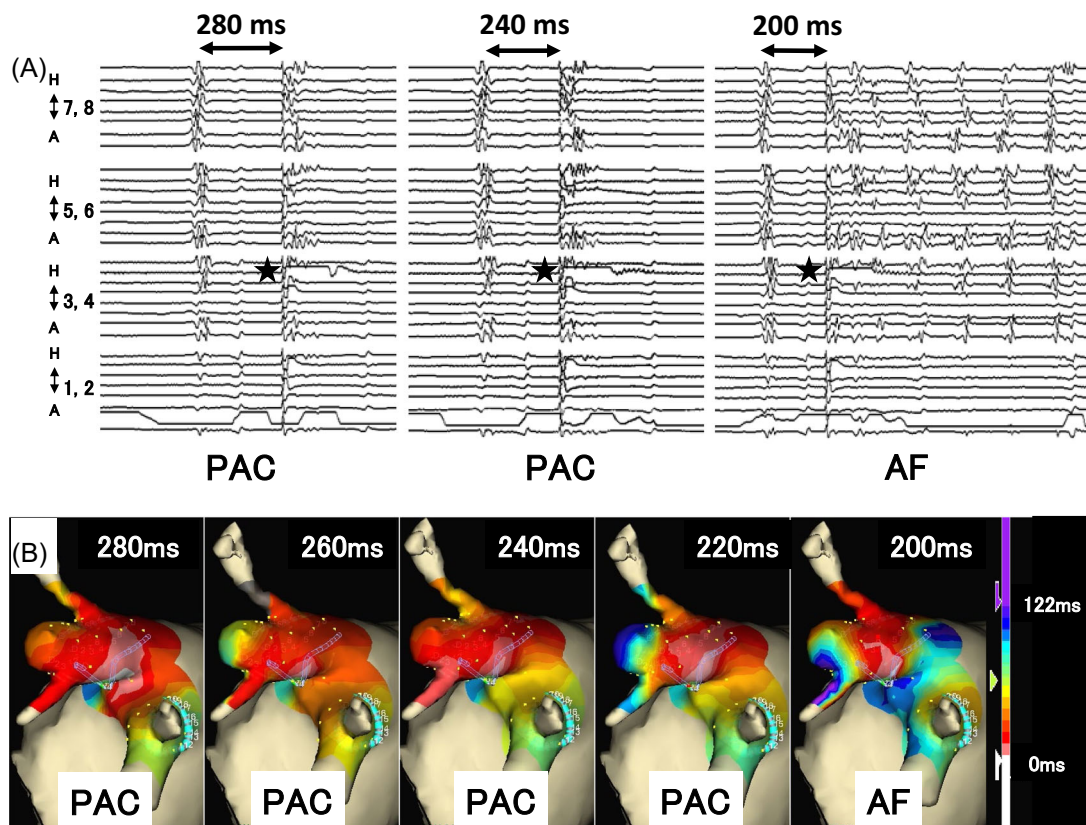
FIGURE 4 Changes of conduction pattern within the pulmonary veins (PVs) in response to the shortening of coupling interval (CI). (A) Changes of intracardiac electrogram. In this case, atrial fibrillation (AF) was induced when a single extra stimulus in the left superior pulmonary vein (LSPV) carina was delivered with a CI of 200 ms. Compared with the intracardiac electrogram with longer CIs, remarkable anisotropic conduction was observed at the onset of AF. (B) Ensite activation mapping of a pacing beat using a 64 electrodes basket catheter in the LSPV and a 20 electrodes circular mapping catheter in the left inferior pulmonary vein (LIPV). Shortening of the CI progressively elicited anisotropic conduction within the PVs, which gave rise to the onset of AF with the CI of 200 ms
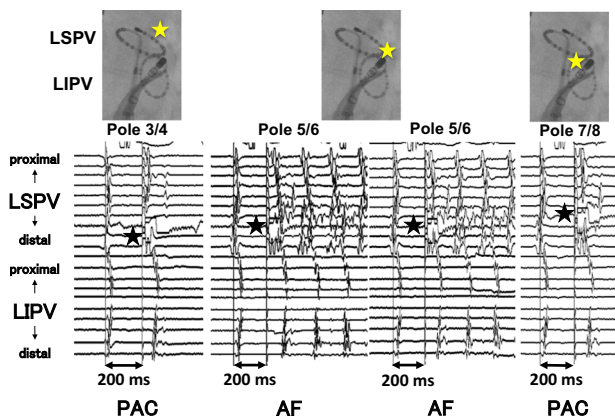

FIGURE 5 Location specificity of atrial fibrillation (AF) induction. In this case, AF was induced reproducibly with the coupling interval (CI) of 200 ms when a single extra stimulus was delivered in the left superior pulmonary vein (LSPV) anterior (pole 5/6). Only bare PAC could be generated even with the same CI in the adjacent area (pole 3/4 or pole 7/8). PAC, premature atrial contraction

AF-inducible sites, significant prolongation of circumferential IPVCT in response to 10 ms shortening of CI was observed preceding the onset of AF (Figures 6A and 7). In contrast, in the AF-noninducible sites, prolongation of IPVCT in response to shortening CI was consistently modest until reaching the pacing ERP (Figures 6B and 7). In quantification, progressive prolongation of circumferential IPVCT in the AF-inducible sites was significantly greater than that in the AF-noninducible sites. In contrast, sequential prolongation of PV-LA conduction time was comparable between the AF-inducible sites and the -noninducible sites (Figure 7).

4 | DISCUSSION

4.1 | Main findings

The main findings of this study were as follows. First, a single extra stimulus in the LSPV could induce AF in 32% of the studied population. Second, onset of AF depended on CI of pacing stimuli and
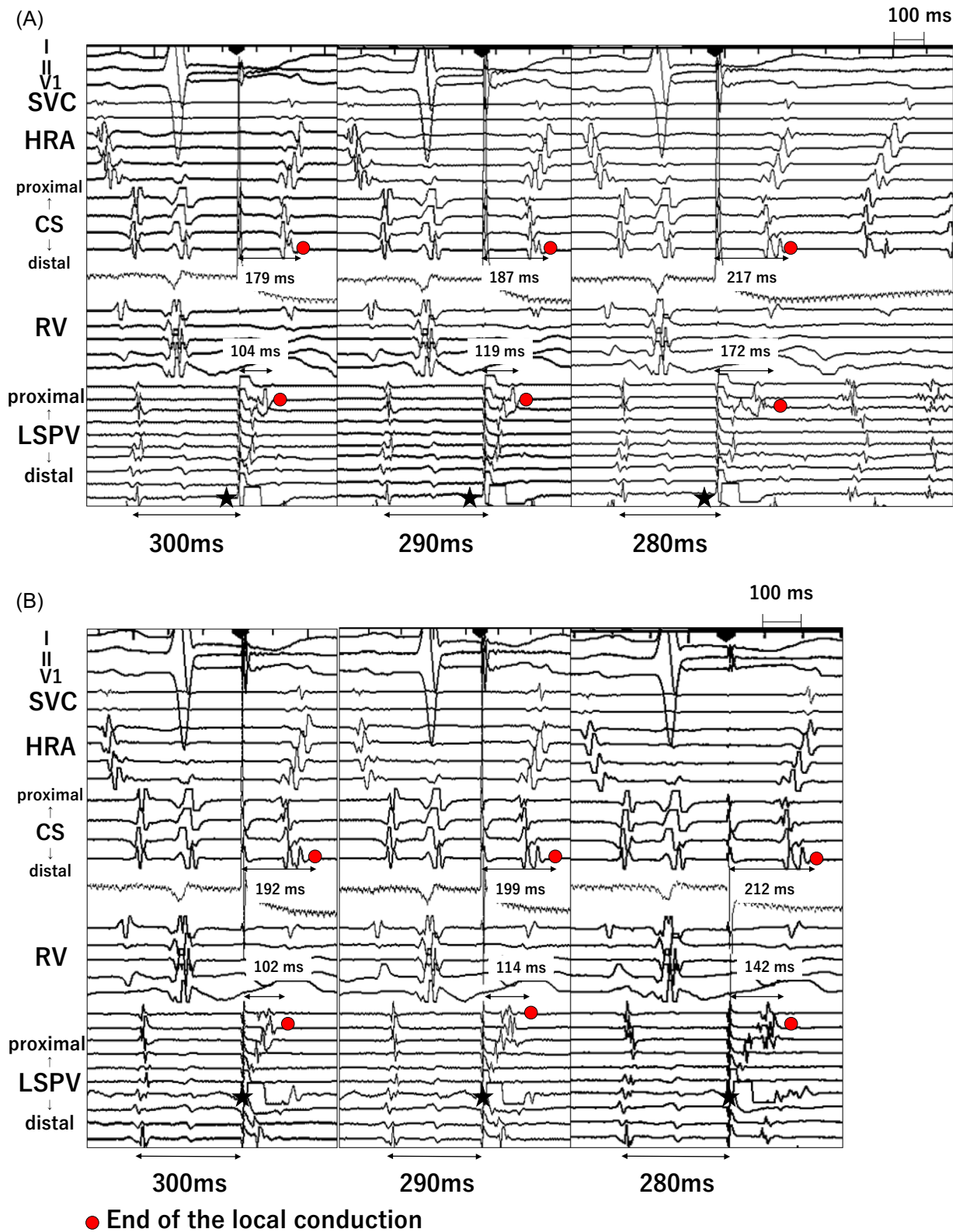
FIGURE 6 Changes of circumferential intra-pulmonary vein (PV) conduction time (IPVCT) and PV-left atrium (PV-LA) conduction time in response to shortening of coupling interval (CI). Red dots indicate the end of the local conduction. (A) When a single extra stimulus was delivered in the atrial fibrillation (AF)-inducible site (left superior pulmonary vein [LSPV] roof), significant prolongation of IPVCT was observed at the onset of AF (from 119 to 172 ms), whereas PV-LA conduction time was prolonged mildly (from 187 to 217 ms). (B) When a single extra stimulus was delivered in the AF-noninducible site (LSPV antero-carina) in the same case as Figure 5A, both IPVCT and PV-LA conduction time consistently showed mild prolongation until the CI reached effective refractory period (ERP) (270 ms in this case)
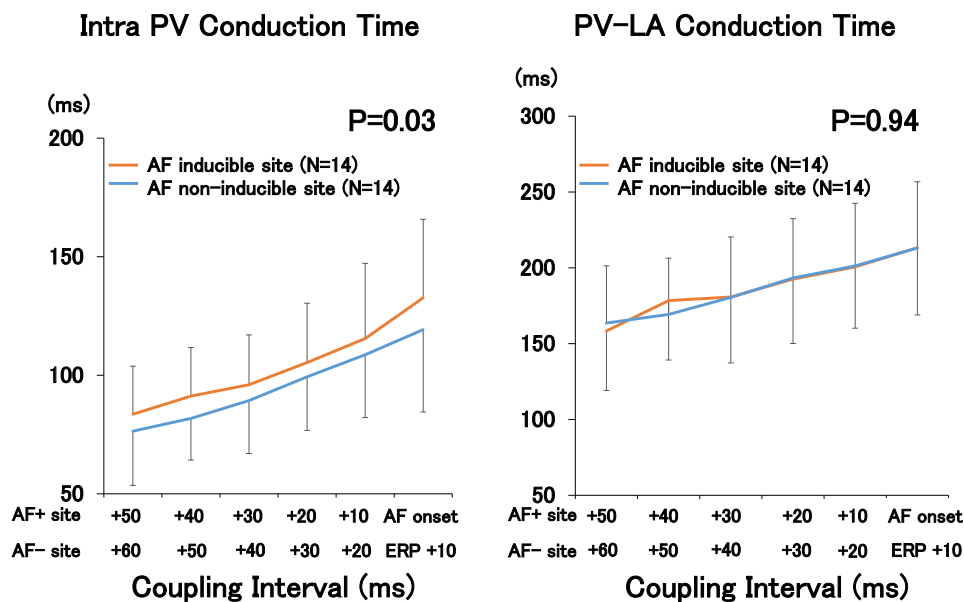
Intra PV Conduction Time

P=0.03

— AF inducible site (N=14)
— AF non-inducible site (N=14)

PV–LA Conduction Time

P=0.94

— AF inducible site (N=14)
— AF non-inducible site (N=14)

FIGURE 7 Sequential changes of conduction time in response to 10 ms shortening of coupling interval (CI). Assessed range is from CI of +50 ms to the CI of atrial fibrillation (AF) onset in the AF-inducible sites, whereas from CI of +60 ms to shortest CI of pulmonary vein (PV) capture (+10 ms of effective refractory period [ERP]) in the AF-noninducible sites

pacing site in the PV with a site-specific reproducibility in each individual. Third, LSPV ERP in the AF-inducible group was shorter than that in the AF-noninducible group. Fourth, LSPV ERP dispersion in the AF-inducible group was greater than that in the AF-noninducible group. Fifth, circumferential intra-PV conduction exhibited decremental properties in response to shortening of CI and prolongation of IPVCT in the AF-inducible site was greater than that in the noninducible site. Finally, prolongation of PV-LA conduction time was comparable between AF-inducible and -noninducible sites. This is the first study demonstrating the ultimate determinant of the AF onset from the PVs.

4.2 | Importance of location and CI of a PV ectopy in the onset of AF

The present study demonstrated that a single ectopic stimulus elicited AF through progressive anisotropic conduction in the PVs. The induction of AF depended on the location of the ectopic stimulus. A short-coupled ectopic stimulus reproducibly elicited AF at a site but not at the adjacent area. Furthermore, the induction of AF depended on the CI of ectopic stimulus. From our observation, it was obvious that an initial anisotropic conduction had a determinant role for the onset of AF, which depended on the location and CI of an ectopic excitation. These findings indicate an important hypothesis that an AF trigger (site) requires a surrounding initiating substrate. Numerous previous studies of AF focused on the difference of electrophysiological properties between patients with and without AF.[3,7] However, no previous study investigated in detail the site-specificity of AF induction.

4.3 | Electrophysiological properties of the PV

We could demonstrate the dynamic conduction pattern changes from a centrifugal activation pattern to anisotropic activation pattern in the PVs in response to the shortening of the CI. PV ERP was shorter and the ERP dispersion was greater in AF-inducible patients than those in AF-noninducible patients. Thus, relatively shorter ERP and its dispersion of myocardial sleeves surrounding an ectopic excitation were associated with initial anisotropic conductions and thus the AF onset from the PVs.

Previous studies of AF investigated the difference of electrophysiological properties between the distal PV and the PV-LA junction.[4,8] In addition, a number of studies discussed the "longitudinal" PV-LA conduction delay;[3,4,7–9] however, no previous study quantitatively discussed the "axial" circumferential intra-PV conduction delay.

The present study demonstrated that circumferential intra-PV conduction delay arose at the onset of AF in response to single extra stimuli, which was more evident at AF-inducible site but not at AF-noninducible sites.

4.4 | Possible mechanisms of anisotropic conduction within the PVs

A previous study compared the voltage map of the PVs among the three groups—control group without AF, paroxysmal AF group, and persistent AF group—and reported that low-voltage area extends as AF stage progresses.[7] Fibrotic tissue alteration may be a possible explanation for ERP dispersion and anisotropic conduction within the

PVs. We previously reported that a non-PV trigger arose in or around the atrial low-voltage area, which may be a similar observation considering the property of AF-triggering sites.[10] Another relevant observation is the relationship between electrophysiological properties in the PVs and myocardial arrangement, reported by Hocini et al.[11] They reported that zones of activation delay and conduction block were related to sudden changes in fiber direction. They also reported that fiber direction at the PV ostium was more complex and disorganized than that at the distal PV. Recently, simulation tests with computational human atrial model revealed that the greatest AF inducibility occurred for cases with circular fibers at the PV ostium.[12,13] Taken together, AF initiation would be determined by an initial anisotropic conduction in relations to location and CI of an ectopic excitation, and surrounding histological backgrounds.

It remains an unsolved argument whether the mechanism of the AF onset is due to reentries or a focal activation with decremental conduction.[8,14,15] It is difficult to confirm a reentry by entrainment study in this field due to the short duration of stable activation pattern. On the other hand, the demonstration of first few beats with identical activation pattern at the onset of AF has been considered as an implication for a reentrant mechanism.[5] Our result also demonstrated a rotational/reentrant activity within a PV using a three-dimensional mapping with a multipolar electrode (Figure 4 and Supplementary Movies). We also implicated reentrant mechanisms within the PVs in patients showing PV bigeminy.[16] In the present study, the first three to four beats following a pacing stimulus showed identical activation pattern preceding the onset of AF, suggesting an initial reentrant mechanism (Figures 4, 5, and 6). This activation pattern gradually collapsed afterward. Thus, we support the hypothesis that the mechanism of the AF onset may be largely due to reentrant mechanisms.

4.5 | Limitations

There are several limitations to be acknowledged in the present study. First, the study population was relatively small due to the single-center, observational study. The present findings need to be confirmed in multicenter studies. Second, the evaluation with voltage mapping within the PVs was not performed. It is conceivable that low-voltage area exists at or around the site where the local functional block arises. It is also conceivable that the long myocardial sleeves are related with AF induction.[17] This hypothesis can be validated by the measurement of myocardial sleeve length using voltage mapping. However, some investigators reported that myocardial sleeve length was longest in patients without AF, and that myocardial sleeve length shortened as AF stage progressed.[7] Third, ERP at the AF onset sites may be overestimated. In the present study, single extra stimuli with shorter CI than that at the AF onset were not delivered considering that the continuation of our study might become impossible due to the persistence of AF as the result of the repetitive AF induction.[18,19] If single extra stimuli were continued to

deliver until CI of a single extra stimulus reached the ERP, the prolongation of IPVCT in the AF-inducible sites may have been greater than that of the present results and the difference in prolongation of IPVCT between AF-inducible and -noninducible sites may have been more remarkable. Fourth, site specificity of AF induction was confirmed only in LSPV. In this study, a series of stimulation protocol added 15–20 min to the total procedure time. Thus, site specificity in other PVs was not investigated in consideration of the overextension of the procedure time.

5 | CONCLUSIONS

Location and CI of an ectopic excitation determine the AF initiation from the PVs, in relations to the surrounding initiating substrates. Dispersion of ERP and dynamic changes of circumferential conduction delay within the PVs may lead to anisotropic conduction and reentry that initiate AF.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Shunsuke Kawai, **Yasushi Mukai**, **Shujiro Inoue**, **Daisuke Yakabe**, **Kazuhiro Nagaoka**, **Kazuo Sakamoto**, and **Susumu Takase**: conception and design or analysis and interpretation of data. **Shunsuke Kawai**, **Yasushi Mukai**, and **Akiko Chishaki**: drafting of the manuscript or revising it critically for important intellectual content. **Hiroyuki Tsutsui**: approval of the manuscript submitted. **Shunsuke Kawai**, **Yasushi Mukai**, **Shujiro Inoue**, **Daisuke Yakabe**, **Kazuhiro Nagaoka**, **Kazuo Sakamoto**, **Susumu Takase**, **Akiko Chishaki**, and **Hiroyuki Tsutsui**: agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the article.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article and its Supplementary Information files.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.