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Electrical PR Interval Variation Predicts New Occurrence of Atrial Fibrillation in Patients With Frequent Premature Atrial Contractions

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Abstract: Atrial fibrillation (AF) is associated with the autonomic nervous system (ANS), and fluctuation of autonomic tone is more prominent in patients with AF. As autonomic tone affects the heart rate (HR), and there is an inverse relationship between HR and PR interval, PR interval variation could be greater in patients with AF than in those without AF. The purpose of this study was to investigate the correlation between PR interval variation and new-onset AF in patients with frequent PACs.

We retrospectively enrolled 207 patients with frequent PACs who underwent electrocardiographs at least 4 times during the follow-up period. The PR variation was calculated by subtracting the minimum PR interval from the maximum PR interval. The outcomes were new occurrence of AF and all-cause mortality during the follow-up period.

During a median follow-up of 8.3 years, 24 patients (11.6%) developed new-onset AF. Univariate analysis showed that prolonged PR interval (PR interval > 200 ms, $P = 0.021$), long PR variation (PR variation > 36.5 ms, $P = 0.018$), and PR variation ($P = 0.004$) as a continuous variable were associated with an increased risk of AF. Cox regression analysis showed that prolonged PR interval (hazard ratio = 3.321, 95% CI 1.064–10.362, $P = 0.039$) and PR variation (hazard ratio = 1.013, 95% CI 1.002–1.024, $P = 0.022$) were independent predictors for new-onset AF. However, PR variation and prolonged PR interval were not associated with all-cause mortality ($P = 0.465$ and 0.774, respectively).

PR interval variation and prolonged PR interval are independent risk factors for new-onset AF in patients with frequent PACs. However we were unable to determine a cut-off value of PR interval variation for new-onset AF.

(*Medicine* 95(14):e3249)

Abbreviations: AF = atrial fibrillation, ANS = autonomic nervous system, ECG = electrocardiography, HR = heart rate, HRV = heart rate variability, HzR = hazard ratio, IQR = interquartile range, miR = microRNA, PACs = premature atrial contractions, PRa = adjusted PR interval, ROC = receiver operating characteristic, RyR2 = type 2 ryanodine receptor.

INTRODUCTION

Atrial fibrillation (AF) is the most common cardiac arrhythmia requiring medical therapy.[1] However, AF is often asymptomatic and is frequently diagnosed for the first time upon admission for stroke management.[2] Indeed, it has been reported that episodes of silent AF were associated with a significantly increased risk of silent cerebral infarct and stroke.[3]

AF is an atrial arrhythmic disease with a multifactorial pathophysiology.[4,5] Several studies have reported that prolonged PR interval and frequent premature atrial contractions (PACs) are associated with AF.[6–9] The autonomic nervous system (ANS) has also been reported to be associated with AF, and both the sympathetic and parasympathetic nervous systems have pro-arrhythmic effects and play an important role in the genesis of AF.[10–14] One prospective study reported that the standard deviation of RR interval before the onset of AF was significantly greater in patients with AF than in controls.[13] This indicates that autonomic fluctuation was more prominent in patients with AF. As autonomic tone affects the heart rate (HR), and there is an inverse relationship between HR and PR interval,[15,16] we hypothesized that the greater the changes in PR interval, the greater the likelihood of AF. The purpose of this study was to investigate the correlation between PR interval variation and new occurrence of AF in patients with frequent PACs.

METHODS

Study Population

We retrospectively enrolled consecutive patients who underwent 24-hour Holter monitoring between April 1999 and June 2008. Frequent PACs were defined as >100 PACs/day during 24-hour Holter monitoring. Among the 2713 patients who underwent 24-hour Holter monitoring, 967 patients with >100 PACs were identified. To estimate sufficient PR interval variation, we enrolled patients who had undergone electrocardiography (ECG) 4 times or more with an interval of at least 1 month during the follow-up period. The exclusion criteria were previously documented AF or atrial flutter, structural heart disease, history of congestive heart failure, high-grade atrioventricular block, pacemaker or implantable cardioverter defibrillator, rheumatic heart disease, moderate to severe heart valve disease, and any mechanical or bioprosthetic heart valve. We also excluded patients who had taken any antiarrhythmic drug within 5 days, and those who had taken amiodarone within the 2 months previous to 24-hour Holter monitoring. This study

received institutional review board approval, and informed consent was waived for this retrospective study.

Analysis of ECGs and 24-Hour Holter Monitoring

We analyzed all 12-lead ECGs that were performed after baseline 24-hour Holter monitoring. The PR interval was automatically measured by the ECG system. If the PR interval was not measured automatically, the PR interval was manually measured using customized software (Cardio Calipers, version 3.3, Iconico Inc, New York, NY) in lead II. All 24-hour Holter monitoring data was analyzed to determine the frequency of PACs and the presence of other arrhythmia, by 2 independent cardiologists. Patients with insufficient 24-hour Holter monitoring data were excluded.

Definitions of Parameters

The maximum PR interval was defined as the longest PR interval among ECGs that were performed from the initial Holter monitoring date to the last follow-up date. The minimum PR interval was defined as the shortest PR interval among ECGs that were performed from the initial Holter monitoring date to the last follow-up date. The PR interval variation was calculated by subtracting the minimum PR interval from the maximum PR interval. A prolonged PR interval was defined as one longer than 200 ms based on initial ECG that was examined when initial Holter monitoring was performed. The PR interval was adjusted using an age- and rate-adjusted formula that was previously reported.[16] Adjusted PR interval was calculated according to the age- and rate-adjusted formula: adjusted PR interval (PRa) = PR + 0.26 (HR − 70) for age group <60 years, and PRa = PR + 0.42 (HR − 70) for age group 60 years or older.

Study Design and End Point

Demographic data, cardiovascular risk factors, medications, and indication for 24-hour ECG monitoring were analyzed by medical records review. We divided all patients into 2 groups according to the best cut-off value of the PR variation-selected receiver operating characteristic (ROC) curve analysis between PR variation and new-onset AF. The primary end point was new occurrence of AF, and the secondary end point was death from any cause. New occurrence of AF and death were evaluated from medical records of our hospital. New occurrence of atrial fibrillation was defined as AF documented by 12-lead electrocardiogram or Holter monitoring during follow-up.

Statistics

Continuous variables are expressed as the mean ± standard deviation or median and interquartile range. Categorical variables are expressed as frequency and percentage. ROC curve analysis was used to select the cut-off value between PR interval variation and new occurrence of AF. To evaluate differences according to new occurrence of AF and all-cause mortality, we used Student's unpaired *t*-test for normally distributed data and a Mann-Whitney test for skewed data. Categorical variables were analyzed with a chi-square test or Fisher's exact test. Cox regression analysis was used to calculate the hazard ratio and 95% confidence interval of new onset AF and all-cause mortality. Calculations were performed using SPSS software (SPSS for Windows, version 20.0, IBM Corp., Armonk, NY). A *P* value of < 0.05 was considered to be significant.

RESULTS

Among the 967 patients with >100 PACs/day, 283 patients were excluded according to the following exclusion criteria: 133 patients had previously documented AF or atrial flutter, 74 patients had structural heart disease, 18 patients had permanent pacemakers, and 58 patients were lost to follow-up. Of these 684 patients, 477 patients who underwent ECG fewer than 4 times were excluded. Ultimately, a total of 207 patients were analyzed in this study.

Baseline Clinical Characteristics of the Study Population

Among the total pool of patients, the mean age was 64.8 ± 12.0 years, and 102 patients (49.3%) were male (Table 1). The median number of PACs was 2640 beats/day (interquartile range [IQR]: 1132–5319 beats/day). The median PR interval was 170 ms (IQR: 154–183 ms), and 21 patients (10.1%) had prolonged PR interval. The mean PR variation was 34.4 ± 25.8 ms; median PR variation was 29.0 ms (IQR: 20.0–41.0 ms). The mean frequency of ECG examination was 5.6 ± 1.6.

Baseline Clinical Characteristics According to New-Onset AF

During a median follow-up of 8.3 years, 24 patients (11.6%) developed new-onset AF. The clinical characteristics according to new-onset AF are summarized in Table 2 (AF [−], group A vs AF [+], group B). Frequency of ECG examination was not significantly different between group A and group B (5.6 ± 1.5 vs 6.0 ± 1.7, *P* = 0.389, respectively). The number of PACs, initial PR interval, maximum PR interval, and minimum PR interval were also not significantly different between the

TABLE 1. Baseline Characteristics of the Study Population

Variable	Total Patients (n = 207)
Male	102 (49.3)
Age (year)	64.8 ± 12.0
Weight (kg)	62.9 ± 11.0
BMI (kg/m²)	24.0 ± 3.4
DM − n (%)	43 (20.8)
Hypertension − n (%)	121 (58.5)
Dyslipidemia − n (%)	17 (8.2)
Coronary artery disease − n (%)	35 (16.9)
PAC (beats/day)	2640 (1132–5319)
PR interval (ms)	170 (154–183)
Prolonged PR interval − n (%)	21 (10.1)
Maximum PR interval (ms)	188 (174–208)
Minimum PR interval (ms)	158 (144–172)
PR variation (ms)	29 (20–41)
Adjusted PR interval (ms)*	168 (155–181)
Adjusted PR variation (ms)	28 (18–39)
Frequency of ECG	5.6 ± 1.6
Duration of follow-up (years)	8.3 (5.3–10.8)

BMI = body mass index, DM = diabetes mellitus, ECG = electrocardiography, HR = heart rate, PAC = premature atrial contraction, PRa = adjusted PR interval.
*Age- and rate-adjusted PR interval formula: PRa = PR + 0.26 (HR − 70) for age group younger than 60 years and PRa = PR + 0.42 (HR − 70) for age group 60 years or older.

TABLE 2. Clinical Characteristics of Patients According to the New Occurrence of Atrial Fibrillation

Variable	AF Occurrence		*P* Value
	No (n = 183)	Yes (n = 24)	
Male	90 (49.2)	12 (50.0)	0.940
Age (year)	64.3 ± 12.2	68.6 ± 9.1	0.135
Weight (kg)	62.8 ± 11.2	63.5 ± 9.5	0.783
BMI (kg/m^2)	24.0 ± 3.5	24.4 ± 2.9	0.596
DM – n (%)	39 (21.3)	4 (16.7)	0.790
Hypertension – n (%)	104 (56.8)	17 (70.8)	0.191
Dyslipidemia – n (%)	16 (8.7)	1 (4.2)	0.699
Coronary artery disease – n (%)	33 (18.0)	2 (8.3)	0.383
PAC (beats/day)	2861 (1146−5510)	1743 (1021−3789)	0.131
Top quartile of PAC – n (%)	47 (25.7)	3 (12.5)	0.156
PR interval (ms)	170 (154−181)	171 (135−203)	0.820
Prolonged PR interval – n (%)	15 (8.2)	6 (25.0)	0.021
Maximum PR interval (ms)	188 (174−205)	195 (175−244)	0.056
Minimum PR interval (ms)	157 (148−171)	160 (132−180)	0.969
PR variation (ms)	28 (19−40)	38 (26−62)	0.004
Long PR variation (PR$_{long}$) – n (%)*	55 (30.1)	13 (54.2)	0.018
Adjusted PR variation (ms)	27 (18−38)	34 (23−61)	0.020
Long adjusted PR variation – n (%)	63 (34.4)	14 (58.3)	0.025
Frequency of ECG	5.6 ± 1.5	6.0 ± 1.7	0.389
Duration of follow-up (y)	8.1 (5.0−10.6)	9.4 (7.1−11.5)	0.023

AF = atrial fibrillation, BMI = body mass index, DM = diabetes mellitus, ECG = electrocardiography, PAC = premature atrial contraction.
*PR$_{long}$ was defined as the PR interval variation >36.5 ms.

2 groups. However, the proportion of prolonged PR interval was significantly higher in group B ($P = 0.021$), and PR interval variation was significantly longer in group B ($P = 0.004$). Duration of follow-up was significantly longer in group B than in group A (9.4 years [IQR: 7.1−11.5 years] vs 8.1 years [IQR: 5.0−10.6 years], $P = 0.023$, respectively).

ROC Analysis of PR Interval Variation According to New-Onset AF

ROC analysis for PR interval variation as a predictor of new-onset AF revealed an area under the curve of 0.679 ($P = 0.004$, Figure 1). A best PR interval variation cut-off value of 36.5 resulted in a sensitivity and specificity for new-onset AF of 54.2% and 69.9%, respectively. Table 3 summarizes the baseline clinical characteristics of the study population according to the cut-off value of PR variation (PR variation ≤ 36.5 ms [PR$_{short}$] vs PR variation > 36.5 ms [PR$_{long}$]). Age, gender, and cardiovascular risk factors were not significantly different between the PR$_{short}$ and PR$_{long}$ groups. The drug history affecting the PR interval also was not significantly different between the 2 groups. The proportion of prolonged PR interval, maximum PR interval, and PR variation were greater in the PR$_{long}$ group ($P = 0.044$, $P < 0.001$, and $P < 0.001$, respectively). The duration of follow-up did not differ between the PR$_{short}$ and PR$_{long}$ groups ($P = 0.340$).

Risk Factors for AF

Univariate analysis showed that prolonged PR interval ($P = 0.021$), PR$_{long}$ ($P = 0.018$), and PR variation ($P = 0.004$) were associated with increased risk of AF. Kaplan−Meier estimates of new-onset AF-free survival according to PR variation are presented in Figure 2 (log rank $P = 0.034$). Cox

regression analysis showed that prolonged PR interval (hazard ratio [HzR] = 3.321, 95% CI 1.064−10.362, $P = 0.039$) and PR variation (HzR = 1.013, 95% CI 1.002−1.024, $P = 0.022$) were independent predictors for the new occurrence of AF (Table 4). However, as a categorical variable, PR$_{long}$ was not associated with new-onset AF in Cox regression analysis (HzR = 1.974, 95% CI 0.845−4.612, $P = 0.116$).
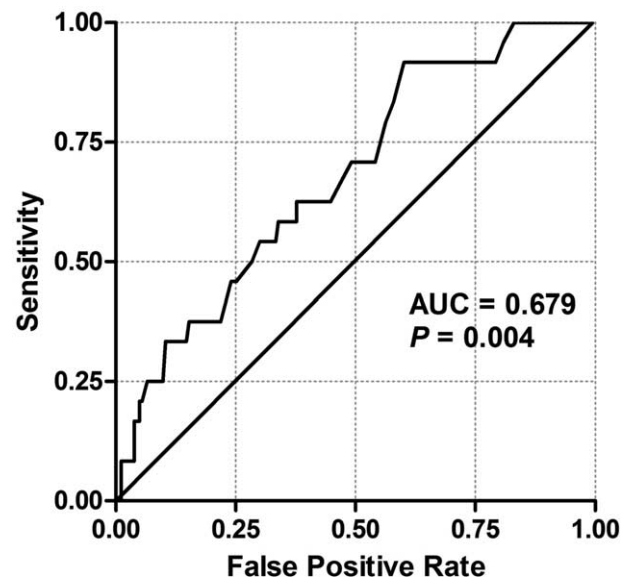
FIGURE 1. ROC curve of PR interval to predict new-onset AF. AF = atrial fibrillation, AUC = area under the curve, ROC = Receiver operating characteristic.

TABLE 3. Baseline Characteristics of the Study Groups

Variable	Short PR Variation (PR$_{short}$, n = 139)	Long PR Variation (PR$_{long}$, n = 68)	P Value
Male	70 (50.4)	32 (47.1)	0.655
Age (year)	63.8 ± 12.5	66.9 ± 10.7	0.090
Weight (kg)	62.9 ± 10.9	62.9 ± 11.1	0.953
BMI (kg/m^2)	23.9 ± 3.3	24.2 ± 3.7	0.628
DM − n (%)	29 (20.9)	14 (20.6)	0.963
Hypertension − n (%)	79 (56.8)	42 (61.8)	0.499
Dyslipidemia − n (%)	12 (8.6)	5 (7.4)	0.753
Coronary artery disease − n (%)	25 (18.0)	10 (14.7)	0.554
Medication			
Beta blocker − n (%)	24 (17.3)	12 (17.6)	0.946
Calcium channel blocker − n (%)	31 (22.3)	15 (22.1)	0.968
ACE inhibitor − n (%)	10 (7.2)	5 (7.4)	1.000
ARB − n (%)	22 (15.8)	12 (17.6)	0.740
Diuretics − n (%)	7 (5.0)	2 (2.9)	0.721
Statin − n (%)	18 (12.9)	6 (8.8)	0.384
Digitalis − n (%)	0 (0.0)	0 (0.0)	−
PR interval (ms)	169 (155−180)	171 (150−186)	0.929
Prolonged PR interval − n (%)	10 (7.2)	11 (16.2)	0.044
PR variation (ms)	22 (16−29)	52 (41−63)	<0.001
Maximum PR interval (ms)	184 (170−192)	210 (189−233)	<0.001
Minimum PR interval (ms)	158 (148−172)	157 (132−173)	0.113
PAC (beats/day)	2224 (1070−5043)	3043 (1296−7406)	0.028
Frequency of ECG	5.4 ± 1.5	6.0 ± 1.7	0.037
Duration of follow-up (y)	7.6 (5.0−10.8)	8.7 (6.3−10.7)	0.340

ACE = angiotensin converting enzyme, ARB = angiotensin receptor blocker, BMI = body mass index, DM = diabetes mellitus, ECG = electrocardiography, PAC = premature atrial contraction.

Risk Factors of All-Cause Mortality

During the follow-up period, the overall mortality rate was 12.6% (26/207). Table 5 presents the clinical characteristics of patients with or without mortality. Patients who developed mortality were significantly older (73.4 ± 8.4 vs 63.6 ± 11.9, $P < 0.001$). Male gender was numerically but not significantly higher in patients with mortality (65.4 vs 47.0%, $P = 0.079$). Prolonged PR interval, PR variation, PR$_{long}$, top quartile of PAC, and new onset AF were not significantly different between patients with and those without mortality. Cox regression analysis showed that age (HzR = 1.111, 95% CI 1.058−1.167, $P < 0.001$) was an independent predictor for all-cause mortality (Table 6).
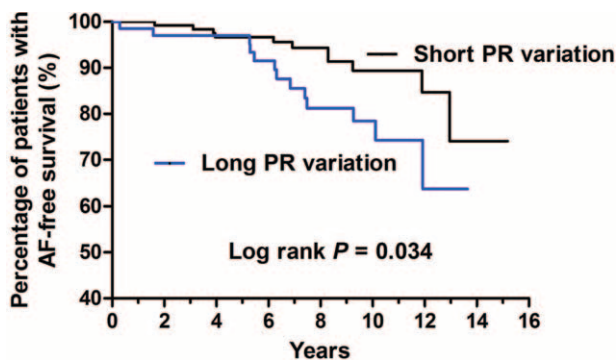
FIGURE 2. Kaplan–Meier estimate of new-onset AF-free survival in patients with frequent PACs. AF = atrial fibrillation, PACs = premature atrial contractions.

DISCUSSION

Atrial electrical and structural remodeling are the most important pathophysiological mechanisms in AF genesis.[4,5] Previous studies have revealed that atrial reverse remodeling can prevent AF development.[17,18] Electrical alterations are

TABLE 4. Multivariate Analysis of the New Occurrence of Atrial Fibrillation

Variable	HR (95% CI)	P Value
Model 1		
Male gender	1.095 (0.437−2.743)	0.847
Age	1.044 (0.996−1.095)	0.073
DM	0.726 (0.235−2.246)	0.579
Hypertension	1.189 (0.452−3.127)	0.726
Prolonged PR interval	3.321 (1.064−10.362)	0.039
PR variation	1.013 (1.002−1.024)	0.022
Top quartile of PAC	0.369 (0.099−1.379)	0.138
Model 2		
Male gender	0.916 (0.379−2.216)	0.846
Age	1.034 (0.988−1.083)	0.145
DM	0.643 (0.213−1.942)	0.433
Hypertension	1.446 (0.550−3.802)	0.455
Prolonged PR interval	3.310 (1.115−9.821)	0.031
Adjusted PR variation	1.022 (1.002−1.043)	0.032
Top quartile of PAC	0.416 (0.123−1.410)	0.159

CI = confidence interval, DM = diabetes mellitus, HR = hazard ratio, PAC = premature atrial contraction.

TABLE 5. Clinical Characteristics of Patients With or Without Mortality

	Death		
Variable	No (n = 181)	Yes (n = 26)	*P* Value
Male	85 (47.0)	17 (65.4)	0.079
Age (y)	63.6 ± 11.9	73.4 ± 8.4	<0.001
Weight (kg)	62.9 ± 10.7	63.0 ± 13.1	0.969
BMI (kg/m^2)	24.0 ± 3.3	23.8 ± 4.0	0.650
DM – n (%)	36 (19.9)	7 (26.9)	0.408
Hypertension – n (%)	103 (56.9)	18 (69.2)	0.233
Dyslipidemia – n (%)	14 (7.7)	3 (11.5)	0.454
Coronary artery disease – n (%)	30 (16.6)	5 (19.2)	0.780
PAC (beats/day)	2461 (1098−5324)	3198 (1433−6119)	0.299
Top quartile of PAC – n (%)	44 (24.3)	6 (23.1)	0.891
PR interval (mm)	169 (154−181)	173 (154−188)	0.323
Prolonged PR interval – n (%)	16 (8.8)	5 (19.2)	0.154
PR variation (ms)	30 (20−41)	27 (23−39)	0.977
Long PR variation (PR$_{long}$) – n (%)[*]	61 (33.7)	7 (26.9)	0.491
New onset AF	20 (11.0)	4 (15.4)	0.514

AF = atrial fibrillation, BMI = body mass index, DM = diabetes mellitus, PAC = premature atrial contraction.
[*]PR$_{long}$ was defined as the PR interval variation >36.5 ms.

related to abnormalities in ionic channel currents and intracellular Ca^{2+} handling.[19,20] Intracellular Ca^{2+} handling in AF patients is related to increased sarcoplasmic Ca^{2+} leakage via the type 2 ryanodine receptor (RyR2), which is a specific molecular target of oxidative stress that is fundamental in the development of AF.[21−23] microRNA (miR)-mediated post-transcriptional regulation of RyR2 is a potential mechanism of paroxysmal AF pathogenesis, and miRs activation and expression patterns are correlated with cardiac electrical and fibrotic remodeling.[24−28] For these reasons, miRs have been used as AF biomarkers in patients treated with catheter ablation.[27−29]

The ANS plays an important role in initiation and maintenance of atrial fibrillation including atrial electrical remodeling.[10,11,30,31] Yang et al[31] showed that increased vagal activity promotes rapid atrial pacing-induced atrial effective refractory periods shortening, which could be blocked by the combination of atropine and a vasoactive intestinal polypeptide antagonist. Most studies that have evaluated the presence of autonomic variation preceding AF used the time and frequency domain parameters of heart rate variability (HRV).[11,13,32,33] HRV is a method that quantitatively measures the balance between sympathetic and parasympathetic activation.[34,35] These studies

revealed that both a linear decrease in mean RR interval and a linear increase in the low/high frequency ratio followed by a sharp decrease immediately before AF were observed before the onset of AF.[32,33] This suggests a primary increase in sympathetic activity followed by a significant shift toward vagal predominance before the onset of AF.

PR interval is also highly affected by the ANS.[36−40] Sympathetic nerve activation results in a decrease of PR interval, whereas parasympathetic nerve activation results in an increase of PR interval. Pirola and Potter[37] reported that the increase in PR interval in response to vagal stimulation is well correlated with vagal stimulation frequency and can be regarded as linear. Therefore, PR interval could vary between instances of performing ECG, according to ANS activation.

The main finding of our study is that the greater the PR interval variation, the greater the risk of AF, in patients with frequent PACs. Even though multivariate analysis including the PR variation expressed as a categorical variable did not find any correlation between long PR variation and new-onset AF, PR variation as a continuous variable was an independent risk factor for new-onset AF. This could be interpreted to indicate that as a greater autonomic variation is noted, the risk of AF increases. In other words, abnormalities in the ANS accentuate PR interval variation and increase the risk of AF. In our study, prolonged PR interval was also associated with new-onset AF. This result is consistent with previous studies.[8,9,41] In fact, our study population is not a general population. All patients had >100 PACs/day. Two prospective studies reported that frequent PACs is an independent risk factor of new-onset AF.[7,42] However, the daily PAC burden was not associated with new-onset AF in our study. In 2 previous studies, frequent PACs were defined as >100 and >720 PACs/day (fourth quartile), respectively.[7,42] The median value of PACs in our study was 2640 PACs/day. This is higher than in the previous studies, indicating that our study population might be susceptible to AF. This susceptibility of our study population to AF might be a cause of the difference in the result about the association between PAC burden and AF development between our and

TABLE 6. Multivariate Analysis of All-Cause Mortality

Variable	HR (95% CI)	*P* Value
Male gender	2.378 (0.976−5.792)	0.056
Age	1.111 (1.058−1.167)	<0.001
DM	1.273 (0.502−3.227)	0.611
Hypertension	0.878 (0.351−2.196)	0.781
Prolonged PR interval	0.843 (0.263−2.700)	0.774
PR variation	0.993 (0.974−1.012)	0.465
Top quartile of PAC	1.426 (0.425−4.787)	0.566

CI = confidence interval, DM = diabetes mellitus, PAC = premature atrial contraction.

previous studies. Regarding all-cause mortality, there was no association between it and PR variation in patients with frequent PACs. There have been several studies reporting an association between the ANS and ventricular arrhythmia causing sudden cardiac death.[43,44] Increased sympathetic or reduced vagal activity plays an important role in ventricular arrhythmia. Priori et al[45] showed that sympathetic nerve stimulation, especially left stellate ganglion stimulation, caused delayed afterdepolarizations, suggesting triggered activity as the mechanism of ventricular arrhythmogenesis. Both sympathetic and vagal activation were necessary to increase PR interval variation. This discrepancy might be one of the potential causes for the lack of relationship seen between PR interval variation and all-cause mortality.

This study has several limitations. First, this study was a retrospective observational study. Therefore, we cannot fully control for confounding factors such as frequency of ECG and daily PAC burden, even though there was no statistically significant difference between patients with and those without new-onset AF. We also could not control for the intervals between ECGs performed during follow-up periods. Second, drug history potentially affecting PR interval was not evaluated at each time that an ECG was performed, although it was not significantly different at the date of initial 24-hour Holter monitoring. For example, although there was no statistically significant difference between the study groups, we could not completely capture patient history of drugs that might affect PR interval, such as beta blockers and calcium channel blockers. Third, although we define the threshold number for frequent PACs base on the previous study, it was arbitrary. Fourth, a 24-hour Holter monitor was used to determine PAC burden. A longer duration of monitoring might be preferable because of day-to-day variability in PAC frequency, especially in the presence of a frequent PAC burden of >100 PACs/day. Whenever feasible, ambulatory monitoring for at least 48 hours is preferable. Fifth, the duration of follow-up was 5 to 11 years in this study. As PR interval increased with age, the PR interval should be adjusted by age. Although we adjusted the PR interval according to HR and age, the adjustment of PR interval might be insufficient because patients were divided into only 2 groups on the basis of age. Sixth, our study patients had >100 PACs/day. Among a total of 2713 patients who underwent 24-hour Holter monitoring during the enrollment period, only 967 patients (35.6%) had >100 PACs/day. This indicates that our study patients are not a general population. Therefore, our findings should be interpreted with caution. Finally, patients that developed AF had an average of an additional 1.3 years of follow-up. Although there is the possibility that this extra follow-up time allowed for the diagnosis of AF, this longer duration of follow-up might be caused by treatment of AF.

In conclusion, our findings indicate that the greater the PR interval variation, the greater the risk of AF in patients with frequent PACs. However, PR interval variation was not associated with all-cause mortality in these patients. Further large-scale, randomized prospective studies are needed to verify our results and to determine the cut-off value of PR interval variation.

REFERENCES

1. Tsang TS, Gersh BJ. Atrial fibrillation: an old disease, a new epidemic. *Am J Med.* 2002;113:432–435.

2. Lin HJ, Wolf PA, Benjamin EJ, et al. Newly diagnosed atrial fibrillation and acute stroke. The Framingham Study. *Stroke.* 1995;26:1527–1530.

3. Marfella R, Sasso FC, Siniscalchi M, et al. Brief episodes of silent atrial fibrillation predict clinical vascular brain disease in type 2 diabetic patients. *J Am Coll Cardiol.* 2013;62:525–530.

4. January CT, Wann LS, Alpert JS, et al. 2014 AHA/ACC/HRS guideline for the management of patients with atrial fibrillation: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and the Heart Rhythm Society. *J Am Coll Cardiol.* 2014;64:e1–e76.

5. Camm AJ, Kirchhof P, Lip GY, et al. Guidelines for the management of atrial fibrillation: the Task Force for the Management of Atrial Fibrillation of the European Society of Cardiology (ESC). *Eur Heart J.* 2010;31:2369–2429.

6. Dewland TA, Vittinghoff E, Mandyam MC, et al. Atrial ectopy as a predictor of incident atrial fibrillation: a cohort study. *Ann Intern Med.* 2013;159:721–728.

7. Chong BH, Pong V, Lam KF, et al. Frequent premature atrial complexes predict new occurrence of atrial fibrillation and adverse cardiovascular events. *Europace.* 2012;14:942–947.

8. Crisel RK, Farzaneh-Far R, Na B, et al. First-degree atrioventricular block is associated with heart failure and death in persons with stable coronary artery disease: data from the Heart and Soul Study. *Eur Heart J.* 2011;32:1875–1880.

9. Cheng S, Keyes MJ, Larson MG, et al. Long-term outcomes in individuals with prolonged PR interval or first-degree atrioventricular block. *JAMA.* 2009;301:2571–2577.

10. Arora R. Recent insights into the role of the autonomic nervous system in the creation of substrate for atrial fibrillation: implications for therapies targeting the atrial autonomic nervous system. *Circ Arrhythm Electrophysiol.* 2012;5:850–859.

11. Lombardi F, Tarricone D, Tundo F, et al. Autonomic nervous system and paroxysmal atrial fibrillation: a study based on the analysis of RR interval changes before, during and after paroxysmal atrial fibrillation. *Eur Heart J.* 2004;25:1242–1248.

12. Patterson E, Po SS, Scherlag BJ, et al. Triggered firing in pulmonary veins initiated by in vitro autonomic nerve stimulation. *Heart Rhythm.* 2005;2:624–631.

13. Amar D, Zhang H, Miodownik S, et al. Competing autonomic mechanisms precede the onset of postoperative atrial fibrillation. *J Am Coll Cardiol.* 2003;42:1262–1268.

14. Rizzo MR, Sasso FC, Marfella R, et al. Autonomic dysfunction is associated with brief episodes of atrial fibrillation in type 2 diabetes. *J Diabetes Complications.* 2015;29:88–92.

15. Atterhog JH, Loogna E. P-R interval in relation to heart rate during exercise and the influence of posture and autonomic tone. *J Electrocardiol.* 1977;10:331–336.

16. Soliman EZ, Rautaharju PM. Heart rate adjustment of PR interval in middle-aged and older adults. *J Electrocardiol.* 2012;45:66–69.

17. Wijffels MC, Kirchhof CJ, Dorland R, et al. Electrical remodeling due to atrial fibrillation in chronically instrumented conscious goats: roles of neurohumoral changes, ischemia, atrial stretch, and high rate of electrical activation. *Circulation.* 1997;96:3710–3720.

18. D'Ascia SL, D'Ascia C, Marino V, et al. Cardiac resynchronisation therapy response predicts occurrence of atrial fibrillation in non-ischaemic dilated cardiomyopathy. *Int J Clin Pract.* 2011;65:1149–1155.

19. Van Wagoner DR, Pond AL, Lamorgese M, et al. Atrial L-type Ca^{2+} currents and human atrial fibrillation. *Circ Res.* 1999;85:428–436.

20. Van Wagoner DR, Pond AL, McCarthy PM, et al. Outward K+ current densities and Kv1.5 expression are reduced in chronic human atrial fibrillation. *Circ Res.* 1997;80:772–781.

21. Santulli G, Marks AR. Essential roles of intracellular calcium release channels in muscle, brain, metabolism, and aging. *Curr Mol Pharmacol.* 2015;8:206–222.

22. Voigt N, Heijman J, Wang Q, et al. Cellular and molecular mechanisms of atrial arrhythmogenesis in patients with paroxysmal atrial fibrillation. *Circulation.* 2014;129:145–156.

23. Xie W, Santulli G, Reiken SR, et al. Mitochondrial oxidative stress promotes atrial fibrillation. *Sci Rep.* 2015;5:11427.

24. Chiang DY, Kongchan N, Beavers DL, et al. Loss of microRNA-106b-25 cluster promotes atrial fibrillation by enhancing ryanodine receptor type-2 expression and calcium release. *Circ Arrhythm Electrophysiol.* 2014;7:1214–1222.

25. Burstein B, Nattel S. Atrial fibrosis: mechanisms and clinical relevance in atrial fibrillation. *J Am Coll Cardiol.* 2008;51:802–809.

26. Nattel S, Burstein B, Dobrev D. Atrial remodeling and atrial fibrillation: mechanisms and implications. *Circ Arrhythm Electrophysiol.* 2008;1:62–73.

27. Santulli G, Iaccarino G, De Luca N, et al. Atrial fibrillation and microRNAs. *Front Physiol.* 2014;5:15.

28. Sardu C, Santamaria M, Paolisso G, et al. microRNA expression changes after atrial fibrillation catheter ablation. *Pharmacogenomics.* 2015;16:1863–1877.

29. McManus DD, Tanriverdi K, Lin H, et al. Plasma microRNAs are associated with atrial fibrillation and change after catheter ablation (the miRhythm study). *Heart Rhythm.* 2015;12:3–10.

30. Zhang L, Po SS, Wang H, et al. Autonomic remodeling: how atrial fibrillation begets atrial fibrillation in the first 24 hours. *J Cardiovasc Pharmacol.* 2015;66:307–315.

31. Yang D, Xi Y, Ai T, et al. Vagal stimulation promotes atrial electrical remodeling induced by rapid atrial pacing in dogs: evidence of a noncholinergic effect. *Pacing Clin Electrophysiol.* 2011;34:1092–1099.

32. Zimmermann M, Kalusche D. Fluctuation in autonomic tone is a major determinant of sustained atrial arrhythmias in patients with focal ectopy originating from the pulmonary veins. *J Cardiovasc Electrophysiol.* 2001;12:285–291.

33. Bettoni M, Zimmermann M. Autonomic tone variations before the onset of paroxysmal atrial fibrillation. *Circulation.* 2002;105:2753–2759.

34. Malik M. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation.* 1996;93:1043–1065.

35. Goldberger JJ. Sympathovagal balance: how should we measure it? *Am J Physiol.* 1999;276:H1273–H1280.

36. Spear JF, Moore EN. Influence of brief vagal and stellate nerve stimulation on pacemaker activity and conduction within the atrioventricular conduction system of the dog. *Circ Res.* 1973;32:27–41.

37. Pirola FT, Potter EK. Vagal action on atrioventricular conduction and its inhibition by sympathetic stimulation and neuropeptide Y in anaesthetised dogs. *J Auton Nerv Syst.* 1990;31:1–12.

38. Victor J, Foussard C, Laporte J, et al. Influence of the autonomic nervous system on the normal and pathological atrioventricular node. *Arch Mal Coeur Vaiss.* 1987;80:1471–1477.

39. Alboni P, Pirani R, Paparella N, et al. Role of the autonomic nervous system in the genesis of first and second degree atrioventricular nodal block. *Eur Heart J.* 1986;7:952–960.

40. Sapire DW, Shah JJ, Black IF. Prolonged atrioventricular conduction in young children and adolescents. The role of increased vagal tone. *S Afr Med J.* 1979;55:669–673.

41. Schnabel RB, Sullivan LM, Levy D, et al. Development of a risk score for atrial fibrillation (Framingham Heart Study): a community-based cohort study. *Lancet.* 2009;373:739–745.

42. Binici Z, Intzilakis T, Nielsen OW, et al. Excessive supraventricular ectopic activity and increased risk of atrial fibrillation and stroke. *Circulation.* 2010;121:1904–1911.

43. Schwartz PJ, Vanoli E, Stramba-Badiale M, et al. Autonomic mechanisms and sudden death. New insights from analysis of baroreceptor reflexes in conscious dogs with and without a myocardial infarction. *Circulation.* 1988;78:969–979.

44. Vaseghi M, Shivkumar K. The role of the autonomic nervous system in sudden cardiac death. *Prog Cardiovasc Dis.* 2008;50:404–419.

45. Priori SG, Mantica M, Schwartz PJ, et al. Delayed after depolarizations elicited in vivo by left stellate ganglion stimulation. *Circulation.* 1988;78:178–185.