

RESEARCH ARTICLE

# Multiscale Entropy of the Heart Rate Variability for the Prediction of an Ischemic Stroke in Patients with Permanent Atrial Fibrillation

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## Abstract

### Background

Atrial fibrillation (AF) is a significant risk factor for ischemic strokes, and making a robust risk stratification scheme would be important. Few studies have examined whether nonlinear dynamics of the heart rate could predict ischemic strokes in AF. We examined whether a novel complexity measurement of the heart rate variability called multiscale entropy (MSE) was a useful risk stratification measure of ischemic strokes in patients with permanent AF.

### Methods and Results

We examined 173 consecutive patients (age 69±11 years) with permanent AF who underwent 24-hour Holter electrocardiography from April 2005 to December 2006. We assessed several frequency ranges of the MSE and CHA<sub>2</sub>DS<sub>2</sub>-VASc score (1 point for congestive heart failure, hypertension, diabetes, vascular disease, an age 65 to 74 years, and a female sex and 2 points for an age ≥75 years and a stroke or transient ischemic attack). We found 22 (13%) incident ischemic strokes during a mean follow up of 3.8-years. The average value of the MSE in the very-low frequency subrange (90–300 s, MeanEn<sub>VL<sub>F2</sub></sub>) was significantly higher in patients who developed ischemic strokes than in those who did not (0.68 ±0.15 vs. 0.60±0.14, P<0.01). There was no significant difference in the C-statistic between the CHA<sub>2</sub>DS<sub>2</sub>-VASc score and MeanEn<sub>VL<sub>F2</sub></sub> (0.56; 95% confidence interval, 0.43–0.69 vs. 0.66; 95% confidence interval, 0.53–0.79). After an adjustment for the age, CHA<sub>2</sub>DS<sub>2</sub>-VASc score, and antithrombotic agent, a Cox hazard regression model revealed that the

Mean $En_{VLF2}$  was an independent predictor of an ischemic stroke (hazard ratio per 1-SD increment, 1.80; 95% confidence interval, 1.17–2.07,  $P < 0.01$ ).

## Conclusion

The Mean $En_{VLF2}$  in 24-hour Holter electrocardiography is a useful risk stratification measure of ischemic strokes during the long-term follow-up in patients with permanent AF.

## Introduction

Atrial fibrillation (AF) is an important risk factor for ischemic strokes, making the prevention of ischemic strokes with oral anticoagulants a mainstay of the current AF clinical practice [1, 2]. To aid in the decisions for prophylaxis and to reduce the exposure of low-risk patients to bleeding complications, a robust risk stratification scheme of the stroke likelihood would be of considerable practical value. To date, as a thromboembolic risk stratification scheme, the CHA<sub>2</sub>DS<sub>2</sub>-VASc score (Congestive Heart failure, hypertension, Age  $\geq 75$  years [doubled], Diabetes, Stroke [doubled], Vascular disease, Age 65–74 years, and Sex category [female sex]) has been established to guide antithrombotic therapy in individuals with AF [3, 4].

During AF, rapid and random atrial impulses create disorganized atrioventricular (AV) nodal conduction and, thus, generate irregular fluctuation in the ventricular response interval (VRI). Such conditions seem to preclude the application of a standard heart rate variability (HRV) analysis [5]. Recently, novel mathematical analyses of the HRV have been developed that provide fresh insight into the abnormalities of the heart rate behavior [6–9], and its association with cardiovascular events [10]. Of these, the multiscale entropy (MSE) analysis was first described by Costa et al. [8, 9] as a method for analyzing the complexity of nonlinear and non-stationary signals in finite length time series. For instance, an MSE analysis allows for the characterization of complex temporal fluctuations inherent in permanent AF [8, 9], as well as provides additional prognostic information in a clinical setting [11]. To date no studies have examined whether the MSE analysis could predict the ischemic strokes in AF patients. We therefore examined whether the MSE represented an independent risk of ischemic strokes in permanent AF patients and compared the predictive value of ischemic strokes with the CHA<sub>2</sub>DS<sub>2</sub>-VASc score.

## Methods

### Patients

We studied consecutive permanent AF patients at our hospital that underwent 24-hour Holter electrocardiograms (ECGs) from April 2005 to December 2006 recorded either as outpatients or during hospitalization. Permanent AF was defined as AF of  $>1$  year of duration, in patients with no evidence of intervening sinus rhythm and in whom there was no plan to restore sinus rhythm [2]. We excluded patients with complete AV block, sustained ventricular tachycardia, ventricular ectopy  $>5\%$  of the 24-hour total beats, cardiac pacemakers, paroxysmal AF, valvular AF or prosthetic heart valves, taking rhythm control drugs, or who had a 24-hour Holter ECG recording in which periods of artifact or noise representing  $>5\%$  of the total monitoring time occurred. Patients who had acute coronary syndrome, strokes or hemodynamic instability in the preceding 6 months, as well as any surgical interventions during the same period, were also excluded. The CHA<sub>2</sub>DS<sub>2</sub>-VASc score was recorded as a baseline measurement of the

stroke risk [3]. The study was approved by the ethics committee of Fujita Health University and conformed to the principles outlined in the Declaration of Helsinki. All patients provided their written informed consent at the time of the Holter recording.

## Holter monitoring and the measurements of the heart rate variability and irregularity

The Holter ECG was recorded with a 2-channel digital recorder (Fukuda Denshi, Tokyo) during the patient's usual daily activities. Patients were asked to submit their diary symptoms and daily activities. ECG signals were digitized at 125 Hz and 12 bits and processed offline using a personal computer equipped with dedicated software. Only recordings with at least 22 hours of data were included in the analysis. The results of the automatic analysis were reviewed, and any errors in the R-wave detection and QRS labeling were edited manually.

We determined the following time domain measures of the variability of the VRI; SD of all the R-R intervals (SDVRI), SD of the 5-minute averages (SDAVRI) to quantify the magnitude of the deviation from the mean, and frequency domain measures including the ultra-low frequency (ULF:  $<0.0033$  Hz), very-low frequency (VLF:  $0.0033$ – $0.04$  Hz), low frequency (LF:  $0.04$ – $0.15$  Hz), and high frequency (HF:  $0.15$ – $0.4$  Hz) bands. As a measure of the irregularity of the VRI, we employed an MSE analysis [8, 9, 12]. The MSE analysis was comprised of two steps: 1) coarse-graining (local averaging) of the observed time series into different time scales; and 2) quantification of the degree of regularity in each coarse-grained time series using a sample entropy [7]. In this study, the sample entropy was calculated using a pattern length  $m$  of 2 and a similarity factor  $r$  of 0.15, and the analyzed VRI time series were interpolated with a linear interpolation and resampled at 2 Hz. To quantify the MSE profile in a plot of the sample entropy vs. frequency ( $\log_{10}$  scale), we calculated the mean value of the sample entropy (MeanEn) and the linear-fitted slope of each scale range corresponding to the frequency-domain measures, namely, the HF (2.5–6.5 s), LF (6.5–25 s), and VLF (25–300 s). In addition, the VLF range was further divided into two subranges, the VLF1 (25–90 s) and VLF2 (90–300 s), respectively. Note that unlike the previous studies [8, 11], the time scale unit of the MSE profile was not in beats but in seconds because the analyses based on the beat scale could be affected by the cardiac rhythm (sinus or non-sinus) and heart rate. In addition, the frequency range of the HRV, such as the HF, LF and VLF, have been defined based on characteristic time scales involved with heart rate modulation. Therefore, we calculated the MSE based on the time scale and compared the MSE measures with the conventional frequency-domain measures. To examine the association between the MSE measures and cardiac autonomic activity, we further determined the degree of concealed conduction of the AV node estimated according to a technique called the Lorenz plot technique (scattering index) [13–15].

## Follow-up and endpoint

Members of the events verification committee who were blinded to the clinical background reviewed the medical records. Valid ischemic strokes were defined as neurological deficits, of sudden onset, that persisted for more than 24 h, and that were not explained by other etiologies (e.g., trauma, infection, or vasculitis). The diagnosis of the ischemic strokes was made by a neurosurgeon either by the use of computed tomography or magnetic resonance imaging. Ischemic strokes included both those due to cardiac emboli and atherothrombotic infarctions.

## Statistical analysis

Differences between the two groups were evaluated using a Student's *t*-test for continuous variables and the  $\chi^2$  test or Fisher's exact probability test for categorical data. The relationship

between the quantitative variables was assessed by a Spearman's rank correlation analysis. A multiple regression analysis was used to test the relationship between clinical variables and MSE. Receiver-operating characteristic curve (ROC) analyses were generated to test the predictive discrimination of significant VRI parameters to identify the association with ischemic strokes during the follow-up. We used Cox models to determine the association of the HRV parameters with the time to an ischemic stroke and we adjusted it for the age, CHA<sub>2</sub>DS<sub>2</sub>-VASc score and the use of antithrombotic agents (warfarin or antiplatelet drug). The hazard ratio (HR) and 95% confidence interval (CI) are given. The cumulative probabilities of an ischemic stroke were estimated as a function of the time using the Nelson-Aalen method and comparisons between the groups were performed using a log-rank test. Training and testing were conducted with a cross-validation technique [16]. The significance of diurnal variation in the MeanEn<sub>VLF2</sub> was tested using a one-way analysis of variance (ANOVA) and Bonferroni post-hoc test. Quantitative data are expressed as the mean±standard deviation (SD) and categorical variables as a percentage. A two-tailed P-value of <0.05 was considered significant. The statistical analyses were performed with JMP 10.0.2 software (SAS Institute, USA) and the R statistical package ([www.r-project.org](http://www.r-project.org)).

## Results

During the recruitment period, 202 consecutive patients were assessed for enrollment eligibility. Twenty-nine patients were excluded according to predetermined clinical criteria.

### Patient characteristics

The baseline clinical characteristics of the 173 patients classified according to the occurrence of ischemic strokes are summarized in [Table 1](#). The mean age of all patients was 69±11 years old (range 30 to 89), of which 35% were ≥75 years old, and 71% were male. There was no significant difference in the CHA<sub>2</sub>DS<sub>2</sub>-VASc score between the 2 groups. Warfarin was being taken by 93 (54%) patients and 30 of those patients were receiving antiplatelets as well. Warfarin was being taken by 88 (53%) in whom the CHA<sub>2</sub>DS<sub>2</sub>-VASc score was ≥1.

### Holter ECG analysis

[Fig 1](#) shows the SampEn of the VRI series of the patients who developed and did not develop ischemic strokes during the observation period. The MeanEn<sub>VLF2</sub> was significantly greater in those who went on to experience a stroke. [Fig 2](#) shows the representative VRIs and their coarse-grained time series from two patients. A patient who developed an ischemic stroke during the follow up had greater values of the mean value of sample entropy of the VLF2 range (MeanEn<sub>VLF2</sub>) and larger fluctuations ([Fig 2A](#)), while a patient that did not experience an ischemic stroke had a lower value of the MeanEn<sub>VLF2</sub> and smaller fluctuations ([Fig 2B](#)).

[Table 2](#) summarizes the HRV parameters. Patients who developed ischemic strokes had higher values of the MeanEn<sub>VLF</sub> (0.76±0.15 vs. 0.68±0.13,  $p = 0.02$ ), MeanEn<sub>VLF1</sub> (0.83±0.16 vs. 0.76±0.14,  $p = 0.04$ ), and MeanEn<sub>VLF2</sub> (0.68±0.15 vs. 0.60±0.14,  $p < 0.01$ ) than those that did not develop ischemic strokes. In the entire patient population, the MeanEn<sub>VLF2</sub> had no substantial correlation to the conventional time- and frequency-domain measures ([Table 3](#)). A multiple regression analysis demonstrated that the MeanEn<sub>VLF2</sub> had a weak association with the sex, but had no association with the comorbidities and concomitant drug therapies ([S1 Table](#)).

### Diurnal variation in the sample entropy

The diurnal variation in the MeanEn<sub>VLF2</sub> is presented in Fig 3. There was a significant diurnal variation in the MeanEn<sub>VLF2</sub> in the study patients (one-way ANOVA, P<0.001). A post-hoc analysis showed that the MeanEn<sub>VLF2</sub> in those that developed ischemic strokes was greater than that in patients that did not develop ischemic strokes during night (19:00, 21:00, 23:00 and 1:00), early morning (5:00 and 9:00), and at 13:00.

### Comparison of the predictive performance

The C-statistics in the patients with and without receiving antithrombotic drugs are summarized in Table 4. There was no significant difference in the C-statistic between the CHA<sub>2</sub>DS<sub>2</sub>-VASc score and MeanEn<sub>VLF2</sub> in all patients, but the C-statistic of the MeanEn<sub>VLF2</sub> was greater than that of the CHA<sub>2</sub>DS<sub>2</sub>-VASc score in the patients that did not receive antithrombotic drugs. Three out of 17 patients with a CHA<sub>2</sub>DS<sub>2</sub>-VASc score of 0 or 1 had an ischemic stroke. The sensitivity and specificity of the mean value of the MeanEn<sub>VLF2</sub> (0.67) was modest at 66.7% and 64.3%, respectively. The 4-fold cross-validation showed that the sensitivity and specificity of the CHA<sub>2</sub>DS<sub>2</sub>-VASc score was 46% and 56%, and MeanEn<sub>VLF2</sub> was 59% and 72%, respectively.

### Time to the event analysis

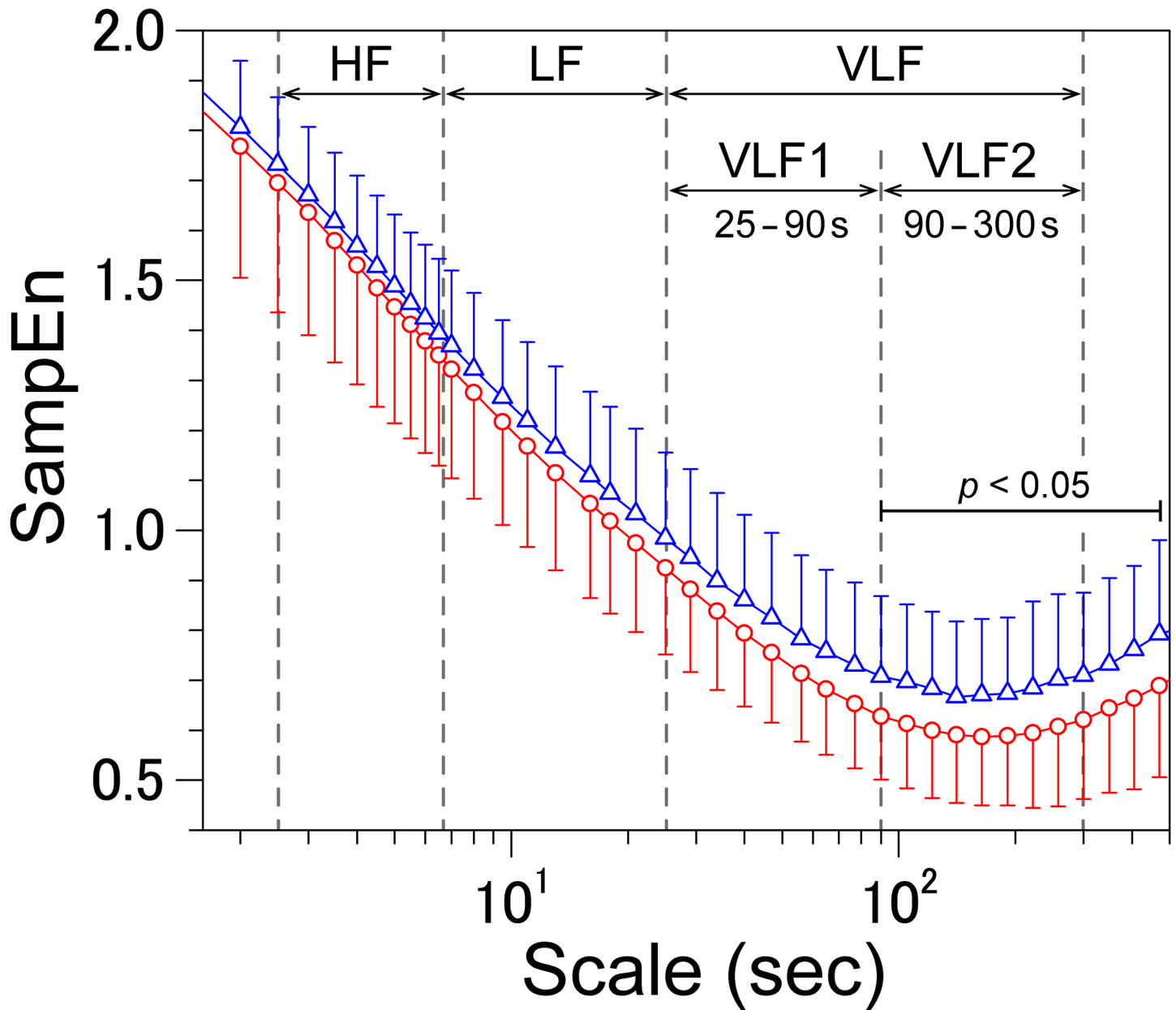
During the mean follow-up of 47±35 months (median 48 months), ischemic strokes were observed in 22 patients for an annualized event rate of 5.8%. The hazard ratios in the study

**Table 1. Baseline clinical characteristics of the patients.**

|  | Ischemic stroke (n = 22) | No ischemic stroke (n = 151) | P-value |
|--|--------------------------|------------------------------|---------|
| Age, years                                   | 71±8                     | 69±11                        | 0.35    |
| Female, n (%)                                | 7 (32)                   | 43 (28)                      | 0.26    |
| Underlying disease, n (%)                    |                          |                              |         |
| Congestive heart failure                     | 9 (41)                   | 56 (37)                      | 0.72    |
| Hypertension                                 | 15(68)                   | 88 (58)                      | 0.38    |
| Diabetes                                     | 1(5)                     | 14 (9)                       | 0.46    |
| Stroke or TIA                                | 9 (41)                   | 40 (26)                      | 0.16    |
| Vascular disease                             | 2 (9)                    | 15 (10)                      | 0.90    |
| CHA <sub>2</sub> DS <sub>2</sub> -VASc score | 3.3±1.6                  | 3.2±1.5                      | 0.38    |
| Echocardiographic findings                   |                          |                              |         |
| Left atrial dimension, mm                    | 45±9                     | 46±11                        | 0.81    |
| Ejection fraction, %                         | 56±9                     | 54±10                        | 0.61    |
| Medications, n (%)                           |                          |                              |         |
| Beta-blocker                                 | 4 (18)                   | 24 (16)                      | 0.79    |
| Digitalis                                    | 8 (36)                   | 65 (43)                      | 0.55    |
| Ca-channel blocker                           | 7 (32)                   | 35 (23)                      | 0.38    |
| ACE inhibitor                                | 7 (32)                   | 37 (25)                      | 0.47    |
| Diuretics                                    | 9 (41)                   | 56 (37)                      | 0.75    |
| Antiplatelet                                 | 5 (23)                   | 41 (27)                      | 0.66    |
| Warfarin                                     | 10 (45)                  | 83 (55)                      | 0.40    |

Ischemic stroke: patients who developed a stroke during the follow-up, No ischemic stroke: patients who remained ischemic stroke free during the follow-up. CHA<sub>2</sub>DS<sub>2</sub>-VASc score (acronym of Chronic heart failure, Hypertension, Age≥75 years, Diabetes, transient ischemic attack or Stroke, Vascular disease, Age≥65 years, and female sex). TIA: transient ischemic attack, ACE: angiotensin II converting enzyme. Data represent the mean±SD or frequency.

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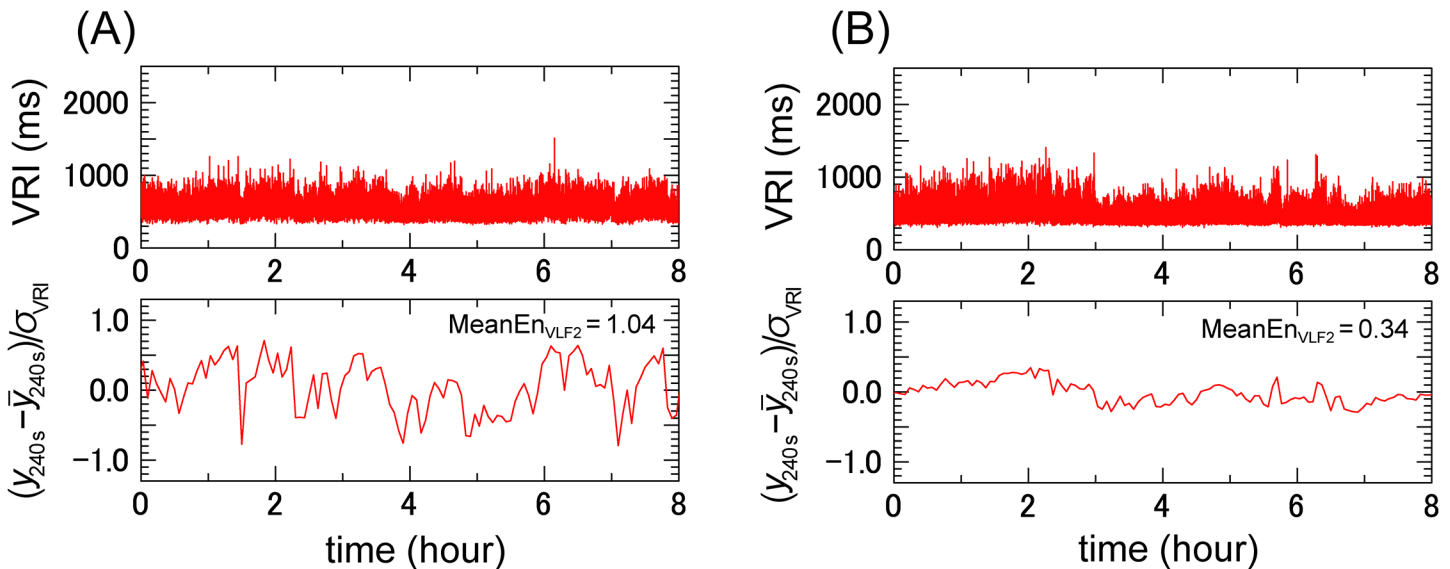


**Fig 1. Multiscale entropy profiles of the ventricular response interval time series.** Comparison between the groups that developed (blue triangle) and did not develop (red triangle) ischemic strokes during the observation period. Significant differences in the SampEn were observed in the VLF2 range. VRI: ventricular response interval, VLF: very low frequency, SampEn: sample entropy. The error bars represent the SD.

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patients are summarized in [Table 5](#). The adjusted Cox hazard regression analyses revealed that in the entire study population, the MeanEn<sub>VLF2</sub> was the best independent predictor of an ischemic stroke (HR 1.80, 95%CI 1.17–2.07) followed by the MeanEn<sub>VLF</sub>, MeanEn<sub>VLF1</sub>, and MeanEn<sub>LF</sub>. In patients who did not take antithrombotic drugs, the MeanEn<sub>VLF2</sub> was the best independent predictor of an ischemic stroke (HR 2.06, 95%CI 1.56–3.48). Nelson-Aalen estimate curves of the patients dichotomized by the mean value of the MeanEn<sub>VLF2</sub> are shown in [Fig 4](#). There was a significant difference in the ischemic stroke rates in all patients and the patients not receiving antithrombotic drugs.





**Fig 2. Ventricular response interval and its coarse-grained time series in patients with and without ischemic strokes.** (A) A 75-year-old male had an ischemic stroke at 2 months after the recording. (B) A 45-year-old male who did not experience an ischemic stroke during 95 months after the recording. The VRI (upper panel) and its coarse-grained time series with a 240 sec (4 min) scale (lower panel) are shown. The coarse-grained time series is rescaled by subtracting its mean and dividing the differences by the SD ( $\delta$ ) of the VRI time series. VRI: ventricular response interval, MeanEn<sub>VLF2</sub>: mean value of sample entropy of the very-low frequency range 2 (90–300 s).

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## Discussion

To the best of our knowledge, this is the first study to examine the predictive value of the novel mathematical method, MSE, for ischemic strokes in patients with permanent AF. Although the conventional HRV failed to predict the ischemic stroke onset, we found that an increased value of the MSE in the very-low frequency subrange (MeanEn<sub>VLF2</sub>) was an independent predictor of an ischemic stroke. This parameter may be a useful risk stratification measure of ischemic strokes in permanent AF patients with an automated analysis of the Holter ECG.

The CHADS<sub>2</sub> score [17] has been used to identify patients with AF at risk for a stroke and systemic embolisms. However, it has limited clinical utility because it is unable to stratify patients at truly low risk of a stroke, who may safely avoid anticoagulation. The CHA<sub>2</sub>DS<sub>2</sub>-VASc score was developed to overcome such limitations by including various risk factors and can better distinguish patients between a low and intermediate risk for a stroke than the CHADS<sub>2</sub> score [4, 18]. In this study we showed that the MeanEn<sub>VLF2</sub> was an independent predictor of an ischemic stroke after an adjustment for the confounders. Further, our strength was that the MeanEn<sub>VLF2</sub> was a useful risk stratification measure of ischemic strokes particularly in patients without taking any antithrombotic drugs. We also showed that the MeanEn<sub>VLF2</sub> had a modest risk stratification power in the patients with a CHA<sub>2</sub>DS<sub>2</sub>-VASc score of 0 and 1.

For sinus rhythm, VRI oscillations in the VLF range are modulated by parasympathetic activity and the endocrine system [19], with a greater power in this frequency range associated with greater parasympathetic activity and a better outcome in patients with cardiovascular diseases [20]. A study of the VRI spectrum in healthy individuals in sinus rhythm and in patients with permanent AF found that the former displayed a  $1/f$  slope over the entire spectrum, while that of the latter displayed a  $1/f$  slope only in the low frequency range (below a frequency corresponding to a period of approximately 200 s) [21]. Above that frequency, the permanent AF patients exhibited a flat white noise like power. We surmised that the similarity of the power spectrum of AF and sinus rhythm in the VLF range permits the power in the VLF in AF

**Table 2. Holter ECG analysis and heart rate variability measurements.**

|                            | Ischemic stroke (n = 22) | No ischemic stroke (n = 151) | P-value |
|----------------------------|--------------------------|------------------------------|---------|
| Mean VRI, ms               | 816±152                  | 811±175                      | 0.91    |
| nVE, beats/24h             | 1319±4786                | 1774±5638                    | 0.72    |
| SDVRI, ms                  | 221±57                   | 230±73                       | 0.59    |
| SDAVRI, ms                 | 119±48                   | 115±55                       | 0.70    |
| ULF, ln (ms <sup>2</sup> ) | 9.23±0.81                | 9.27±1.01                    | 0.87    |
| VLF, ln (ms <sup>2</sup> ) | 8.24±0.76                | 8.28±0.86                    | 0.83    |
| LF, ln (ms <sup>2</sup> )  | 9.12±0.77                | 9.20±0.89                    | 0.70    |
| HF, ln (ms <sup>2</sup> )  | 9.63±0.64                | 9.72±0.75                    | 0.63    |
| Mean En <sub>HF</sub>      | 1.57±0.14                | 1.52±0.24                    | 0.36    |
| Mean En <sub>LF</sub>      | 1.18±0.16                | 1.13±0.19                    | 0.21    |
| Mean En <sub>VLF</sub>     | 0.76±0.15                | 0.68±0.13                    | 0.02    |
| Mean En <sub>VLF1</sub>    | 0.83±0.16                | 0.76±0.14                    | 0.04    |
| Mean En <sub>VLF2</sub>    | 0.68±0.15                | 0.60±0.14                    | <0.01   |
| Slope En <sub>HF</sub>     | -0.82±0.17               | -0.84±0.18                   | 0.68    |
| Slope En <sub>LF</sub>     | -0.71±0.17               | -0.73±0.20                   | 0.77    |
| Slope En <sub>VLF</sub>    | -0.26±0.16               | -0.29±0.18                   | 0.42    |
| Slope En <sub>VLF1</sub>   | -0.51±0.14               | -0.54±0.21                   | 0.52    |
| Slope En <sub>VLF2</sub>   | 0.02±0.20                | -0.02±0.18                   | 0.43    |
| Scattering index           | 381±89                   | 390±117                      | 0.66    |

VRI: ventricular response interval, nVE: number of ventricular ectopy, SDVRI: SD of VRI, SDAVRI: SD of 5-min average VRI, ULF: ultra-low frequency ( $\geq 333$  s) power, VLF1: very-low frequency (25–90 s) power, VLF2: very-low frequency (90–300 s) power, LF: low frequency (6.7–25s) power, HF: high frequency (2.5–6.7s) power, En: sampling entropy. En<sub>VLF2</sub>: sampling entropy of a very-low frequency range (90–300 s). ln: natural logarithm. Data represent the means±SD.

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**Table 3. Relationships between the clinical features and VRI measures.**

|                         | Mean VRI          | SDVRI             | SDAVRI             | ULF                | VLF               | LF                | HF                | Mean En <sub>VLF2</sub> | Scattering index  |
|-------------------------|-------------------|-------------------|--------------------|--------------------|-------------------|-------------------|-------------------|-------------------------|-------------------|
| SDVRI                   | 0.73 <sup>§</sup> |                   |                    |                    |                   |                   |                   |                         |                   |
| SDAVRI                  | 0.45 <sup>§</sup> | 0.79 <sup>§</sup> |                    |                    |                   |                   |                   |                         |                   |
| ULF                     | 0.46 <sup>§</sup> | 0.79 <sup>§</sup> | 0.90 <sup>§</sup>  |                    |                   |                   |                   |                         |                   |
| VLF                     | 0.71 <sup>§</sup> | 0.89 <sup>§</sup> | 0.62 <sup>§</sup>  | 0.72 <sup>§</sup>  |                   |                   |                   |                         |                   |
| LF                      | 0.76 <sup>§</sup> | 0.93 <sup>§</sup> | 0.65 <sup>§</sup>  | 0.68 <sup>§</sup>  | 0.90 <sup>§</sup> |                   |                   |                         |                   |
| HF                      | 0.74 <sup>§</sup> | 0.90 <sup>§</sup> | 0.62 <sup>§</sup>  | 0.64 <sup>§</sup>  | 0.83 <sup>§</sup> | 0.97 <sup>§</sup> |                   |                         |                   |
| Mean En <sub>VLF2</sub> | 0.25 <sup>§</sup> | 0.12              | 0.14               | 0.16 <sup>*</sup>  | 0.23 <sup>†</sup> | 0.15 <sup>*</sup> | 0.14              |                         |                   |
| Scattering index        | 0.78 <sup>§</sup> | 0.93 <sup>§</sup> | 0.65 <sup>§</sup>  | 0.67 <sup>§</sup>  | 0.83 <sup>§</sup> | 0.90 <sup>§</sup> | 0.90 <sup>§</sup> | 0.28 <sup>§</sup>       |                   |
| Age                     | -0.02             | -0.12             | -0.18 <sup>*</sup> | -0.18 <sup>*</sup> | -0.13             | -0.08             | -0.04             | 0.01                    | -0.08             |
| Male sex                | 0.08              | 0.20 <sup>†</sup> | 0.23 <sup>†</sup>  | 0.22 <sup>*</sup>  | 0.25 <sup>†</sup> | 0.18 <sup>*</sup> | 0.20 <sup>*</sup> | 0.16                    | 0.03              |
| Ejection fraction       | 0.23 <sup>*</sup> | 0.25 <sup>*</sup> | 0.17               | 0.13               | 0.22 <sup>*</sup> | 0.25 <sup>*</sup> | 0.20              | 0.08                    | 0.27 <sup>§</sup> |
| Left atrial dimension   | 0.11              | 0.05              | -0.02              | -0.01              | 0.02              | 0.07              | 0.08              | -0.04                   | 0.05              |

Data represent Spearman's rank correlation coefficients. The abbreviations are as in Tables 1 and 2.

\* P<0.05

† P<0.01

§ P<0.001.

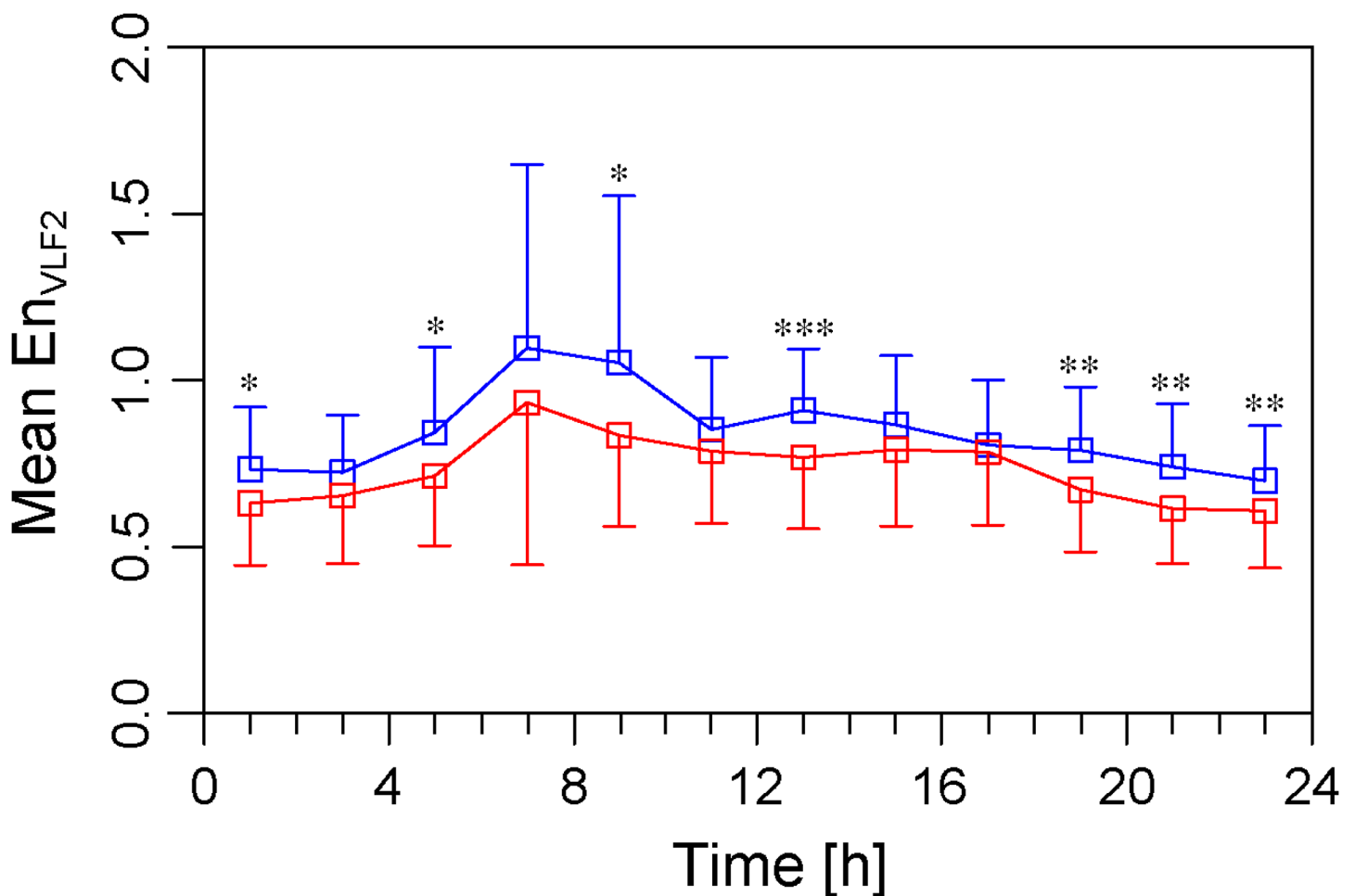
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patients to be interpreted as reflecting parasympathetic activity as has been shown for sinus rhythm.

In the previous MSE analysis for permanent AF [8], the MSE profile had been estimated only for a relatively short scale range (<20 beats), corresponding to the HF range and LF range in the conventional HRV analysis. In that short scale range, however, the MSE profile of AF patients only showed a white noise-like behavior as stated above, and suggests that the VRI has no temporal correlations in AF, unlike sinus rhythm. However, by calculating the MSE profile over a wider range of scales that included the VLF range, we were able to confirm the presence of long time scale VRI correlations in AF [21] and quantify them to differentiate patients who developed ischemic strokes from those who did not. This technique demonstrated that AF patients who later developed ischemic strokes displayed higher entropy values.

In this study we found that the MeanEn<sub>VLF2</sub> had a weak but significant positive relationship with the scattering index, suggesting that the MeanEn<sub>VLF2</sub> is likely to be correlated with a higher vagal tone [14, 15]. The electrical remodeling in AF may shorten the atrial refractoriness, thereby increasing the number and frequency of fibrillatory waves, which could increase the MSE by enhancing concealed conduction within the AV node [22, 23]. Also, shown in



**Fig 3. Diurnal variation in the sample entropy in the VLF2 subrange.** The MeanEn<sub>VLF2</sub> for a 4-hour period was plotted every 2 hours, where the center of the interval was assigned as the time. The blue rectangles denote the patients with incident ischemic strokes and the red rectangles denote the patients that did not develop ischemic strokes. MeanEn<sub>VLF2</sub>: mean value of sample entropy of the very-low frequency range 2 (90–300 s).

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**Table 4. C-statistics.**

|                                     | CHA <sub>2</sub> DS <sub>2</sub> -VASc | MeanEn <sub>VLF2</sub> | P-value |
|-------------------------------------|--|------------------------|---------|
| All patients (n = 173)              | 0.56 (0.43–0.69)                       | 0.66 (0.53–0.79)       | 0.07    |
| No antithrombotic treatment(n = 64) | 0.54 (0.35–0.73)                       | 0.75 (0.58–0.93)       | <0.05   |
| Antithrombotic treatment(n = 109)   | 0.68 (0.53–0.83)                       | 0.59 (0.39–0.79)       | 0.31    |

Abbreviations are as in Tables 1 and 2.

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Fig 2, the large baseline drift that appeared in the patients with strokes meant a clustered appearance of relatively long VRIs, which then created more episodes of longer durations of the blood remaining in the atrium, which may have accelerated the thrombus formation in permanent AF, but this hypothesis deserves further study [24].

There is a diurnal variation in the onset of strokes with the greatest prevalence during sleep and the early morning hours [25, 26]. A similar circadian variability is observed in likely thrombogenic precipitants including the blood pressure, vasoconstrictor tone, blood viscosity, and platelet aggregability [27–30]. In this study we observed that patients who developed ischemic strokes exhibited an increased MeanEn<sub>VLF2</sub> compared to those that did not develop ischemic strokes during the night and early morning hours. Also, we observed postprandial elevation in the MeanEn<sub>VLF2</sub> (9:00, 13:00, and 19:00). There was no significant difference in the prevalence of diabetes between two groups, but postprandial elevation in MeanEn<sub>VLF2</sub> may associate with impaired postprandial autonomic nervous system responsiveness. Demonstrations of these associations do not establish a cause-effect relation, but an increased MeanEn<sub>VLF2</sub> may provide insight into the mechanisms precipitating ischemic strokes in AF.

### Study limitations

This study was an observational, small cohort of Japanese patients at a single institution, which may constitute a selection bias. The presence of diabetic patients or medications may have

**Table 5. Cox proportional hazards regression analysis.**

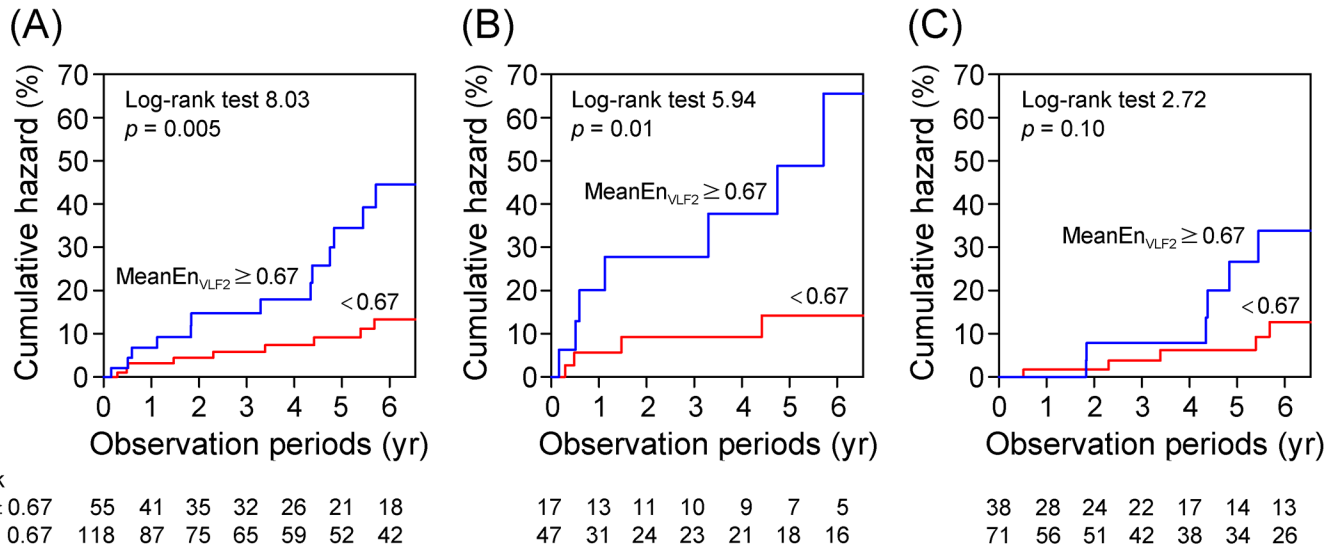
|                          | All patients (n = 173) |         | No antithrombotic drug <sup>a</sup> (n = 64) |         | Antithrombotic drug <sup>b</sup> (n = 109) |         |
|--------------------------|------------------------|---------|--|---------|--|---------|
|                          | HR (95%CI)             | P-value | HR (95%CI)                                   | P-value | HR (95%CI)                                 | P-value |
| Mean En <sub>HF</sub>    | 1.56 (0.96–2.69)       | 0.07    | 1.56 (0.73–3.83)                             | 0.27    | 1.47 (0.81–2.95)                           | 0.21    |
| Mean En <sub>LF</sub>    | 1.58* (1.00–2.56)      | 0.04    | 1.10 (0.75–1.63)                             | 0.32    | 1.68 (0.94–3.11)                           | 0.08    |
| Mean En <sub>VLF</sub>   | 1.74* (1.14–2.62)      | 0.01    | 1.99 (1.04–3.67)                             | 0.03    | 1.65 (0.92–2.95)                           | 0.09    |
| Mean En <sub>VLF1</sub>  | 1.61* (1.06–2.44)      | 0.02    | 1.69 (0.83–3.59)                             | 0.15    | 1.66 (0.96–2.85)                           | 0.051   |
| Mean En <sub>VLF2</sub>  | 1.80* (1.17–2.07)      | <0.01   | 2.06* (1.56–3.48)                            | 0.01    | 1.58 (0.83–3.08)                           | 0.16    |
| Slope En <sub>HF</sub>   | 1.34 (0.10–9.47)       | 0.80    | 0.73 (0.28–1.57)                             | 0.45    | 1.31 (0.80–1.80)                           | 0.22    |
| Slope En <sub>LF</sub>   | 0.57 (0.04–6.72)       | 0.66    | 0.90 (0.36–2.04)                             | 0.81    | 0.94 (0.51–1.81)                           | 0.93    |
| Slope En <sub>VLF</sub>  | 1.05 (0.06–1.50)       | 0.80    | 1.83(0.89–4.21)                              | 0.10    | 0.62 (0.31–1.26)                           | 0.18    |
| Slope En <sub>VLF1</sub> | 0.99 (0.64–1.58)       | 0.99    | 1.29 (0.69–2.58)                             | 0.42    | 0.71 (0.36–1.43)                           | 0.33    |
| Slope En <sub>VLF2</sub> | 2.70 (0.89–88.8)       | 0.57    | 2.16* (1.03–4.84)                            | 0.04    | 0.64 (0.33–1.25)                           | 0.19    |

a: adjusted by age and the CHA<sub>2</sub>DS<sub>2</sub>-VASc score in patients who did not receive antithrombotic drugs.

b: adjusted by age, CHA<sub>2</sub>DS<sub>2</sub>-VASc score and antithrombotic drugs (either warfarin or antiplatelet drug).

\* denotes P<0.05. HR = hazard ratio per 1-SD increment. CI: confidence interval. The other abbreviations are as in Tables 1 and 2.

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**Fig 4. Nelson-Aalen estimate of the ischemic strokes.** (A) All patients (n = 173), (B) Patients not taking antithrombotic drugs (n = 64), and (C) Patients taking antithrombotic drugs (n = 109).

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influenced the activity of the autonomic nervous system. Other important limitations were the underuse of warfarin (only 53% of patients with a CHA<sub>2</sub>DS<sub>2</sub>-VASc score  $\geq 1$ ) and the work was probably underpowered for defining the correlation between the MeanEn<sub>VLF2</sub> and ischemic strokes in anticoagulated patients. Moreover, the international normalized ratio values in the patients that were anticoagulated who experienced ischemic strokes have not been reported. The differences observed between the groups in the MSE parameters were not large and this may limit the clinical value of the result. Although the findings have internal validity in this cohort, further research is needed in larger samples to determine the external validity of our findings.

### Conclusion

Patients with AF exhibit largely diverse disease characteristics and continue to be at high risk for strokes. The MeanEn<sub>VLF2</sub> may be a useful marker for prediction of ischemic strokes in permanent AF patients with an automated analysis of the Holter ECG.

### Supporting Information

**S1 Data. Data set file.**

(XLSX)

**S1 Table. Multiple regression analyses of the MeanEn\_VLF2.** R<sup>2</sup> = 0.28. En: sampling entropy, VLF2: very-low frequency (90–300 s) power, TIA: transient ischemic attack, ACE: angiotensin II converting enzyme.

(DOC)

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## Author Contributions

Conceived and designed the experiments: EW KK JH YY. Performed the experiments: MY TI YS MH. Analyzed the data: KK JH YY. Contributed reagents/materials/analysis tools: JI. Wrote the paper: EW KK. Final approval of the version to be published: YO.

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