ORIGINAL RESEARCH ARTICLE

Abnormal P-wave terminal force in lead V_1 is a marker for atrial electrical dysfunction but not structural remodelling

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Abstract

Aims   There is a lack of diagnostic and therapeutic options for patients with atrial cardiomyopathy and paroxysmal atrial fibrillation. Interestingly, an abnormal P-wave terminal force in electrocardiogram lead V_1 ($PTFV_1$) has been associated with atrial cardiomyopathy, but this association is poorly understood. We investigated $PTFV_1$ as a marker for functional, electrical, and structural atrial remodelling.

Methods and results   Fifty-six patients with acute myocardial infarction and 13 kidney donors as control cohort prospectively underwent cardiac magnetic resonance imaging to evaluate the association between $PTFV_1$ and functional remodelling (atrial strain). To further investigate underlying pathomechanisms, right atrial appendage biopsies were collected from 32 patients undergoing elective coronary artery bypass grafting. $PTFV_1$ was assessed as the product of negative P-wave amplitude and duration in lead V_1 and defined as abnormal if $\geq$4000 ms*μV. Activity of cardiac Ca/calmodulin-dependent protein kinase II (CaMKII) was determined by a specific HDAC4 pull-down assay as a surrogate for electrical remodelling. Atrial fibrosis was quantified using Masson's trichrome staining as a measure for structural remodelling. Multivariate regression analyses were performed to account for potential confounders. A total of 16/56 (29%) of patients with acute myocardial infarction, 3/13 (23%) of kidney donors, and 15/32 (47%) of patients undergoing coronary artery bypass grafting showed an abnormal $PTFV_1$. In patients with acute myocardial infarction, left atrial (LA) strain was significantly reduced in the subgroup with an abnormal $PTFV_1$ (LA reservoir strain: 32.28 ± 12.86% vs. 22.75 ± 13.94%, $P = 0.018$; LA conduit strain: 18.87 ± 10.34% vs. 10.17 ± 8.26%, $P = 0.004$). Abnormal $PTFV_1$ showed a negative correlation with LA conduit strain independent from clinical covariates (coefficient B: −7.336, 95% confidence interval −13.577 to −1.095, $P = 0.022$). CaMKII activity was significantly increased from (normalized to CaMKII expression) 0.87 ± 0.17 to 1.46 ± 0.15 in patients with an abnormal $PTFV_1$ ($P = 0.047$). This increase in patients with an abnormal $PTFV_1$ was independent from clinical covariates (coefficient B: 0.542, 95% confidence interval 0.057 to 1.027, $P = 0.031$). Atrial fibrosis was significantly lower with 12.32 ± 1.63% in patients with an abnormal $PTFV_1$ (vs. 20.50 ± 2.09%, $P = 0.006$), suggesting $PTFV_1$ to be a marker for electrical but not structural remodelling.

Conclusions   Abnormal $PTFV_1$ is an independent predictor for impaired atrial function and for electrical but not for structural remodelling. $PTFV_1$ may be a promising tool to evaluate patients for atrial cardiomyopathy and for risk of atrial fibrillation.

Introduction

Atrial fibrillation (AF) is a widespread disease with an increasing prevalence and a great socio-economic relevance, but treatment options and early diagnostic tools are still limited.[1,2] Atrial cardiomyopathy summarizes pathological functional, electrical, and structural remodelling in the atria.[2] In this context, atrial fibrosis as an unspecific reaction to many pathologic stimuli such as increased wall stress or inflammation, and atrial fibrosis constitutes a key finding in atrial cardiomyopathy.[2] Inflammation and reactive oxygen species production lead to activation of the interleukin-6-related MEK5-ERK5 and STAT-3 pathways, which are linked to fibrotic myocardial remodelling.[2] Arterial hypertension activates the renin–angiotensin–aldosterone axis, which then activates profibrotic pathways.[2] Fibrotic changes in the atrial wall structure are already found in early stages of lone paroxysmal AF, when atrial function and electrical conduction assessed with electrocardiogram (ECG) and echocardiography are still unchanged. Progressing fibrosis can lead to conduction abnormalities and is the structural correlate for re-entry mechanisms. Furthermore, loss of viable myocytes and increased wall tissue stiffness due to excessive fibrosis eventually reduce atrial function.[2] Atrial fibrosis therefore is a hallmark of atrial cardiomyopathy.[2]

Interestingly, atrial dysfunction measured volumetrically as increased size or reduced atrial ejection fraction has been associated with adverse cardiovascular outcome such as higher risk of stroke,[3] new onset of AF,[4] and higher mortality after myocardial infarction (MI).[2,5] Importantly, up to one-fourth of all strokes is considered cryptogenic with no apparent cause.[6] Many of these strokes are cardioembolic, but paroxysmal AF may only be detected after extensive long-term monitoring.[6] Therefore, early identification of atrial remodelling as a precursor of AF is of utmost importance to identify patients at risk for stroke. Unfortunately, current diagnostic tools are limited. In contrast to standard evaluation of atrial volumetry, analysis of myocardial deformation via strain analysis allows for more detailed assessment of atrial function.[7] Also, reduced strain correlates with atrial fibrofatty remodelling.[8] Despite these advantages, it is not yet routinely employed in clinical practice due to the limited availability of the necessary hardware and software. Interestingly, several novel ECG indices for atrial cardiomyopathy have been proposed. Among these, an abnormal P-wave terminal force in lead V_1 ($PTFV_1$) has been shown to be an independent predictor for AF, stroke, and cardiac death or hospitalization for heart failure in patients with prior MI.[5,9,10] Abnormal $PTFV_1$ is currently evaluated as a risk marker for secondary prevention of patients with cryptogenic stroke.[11] However, the underlying mechanisms for the development of abnormal $PTFV_1$ and the consequences for atrial function are unknown. Interestingly, an increased Ca/calmodulin-dependent protein kinase II (CaMKII)-dependent dysregulation of cardiomyocytes

ion homeostasis has already been associated with atrial pathologies.[1,2,12–14]

The aim of the present study was to evaluate the link between abnormal $PTFV_1$, atrial strain, and atrial CaMKII-dependent pro-arrhythmic activity in high-risk patients with cardiovascular disease. This is of emerging interest because several CaMKII inhibitors are under preclinical consideration and have already been successfully tested in human myocardium.[13]

Methods

An extended description of all materials and methods can be found in the Supporting Information. To avoid the possibility of unintentional distribution of private patient data, patient information can only be made available after written informed consent has been given by each patient for a specific request.

Study approval and design

For this study, patient data, cardiac magnetic resonance imaging (CMR), and specimens of two distinct studies and one control cohort were used. All studies were approved by the local ethics committee and are in accordance with the Declaration of Helsinki (first released in 1964, most recent revision 2013). Each patient has given his written informed consent prior to inclusion in this study.
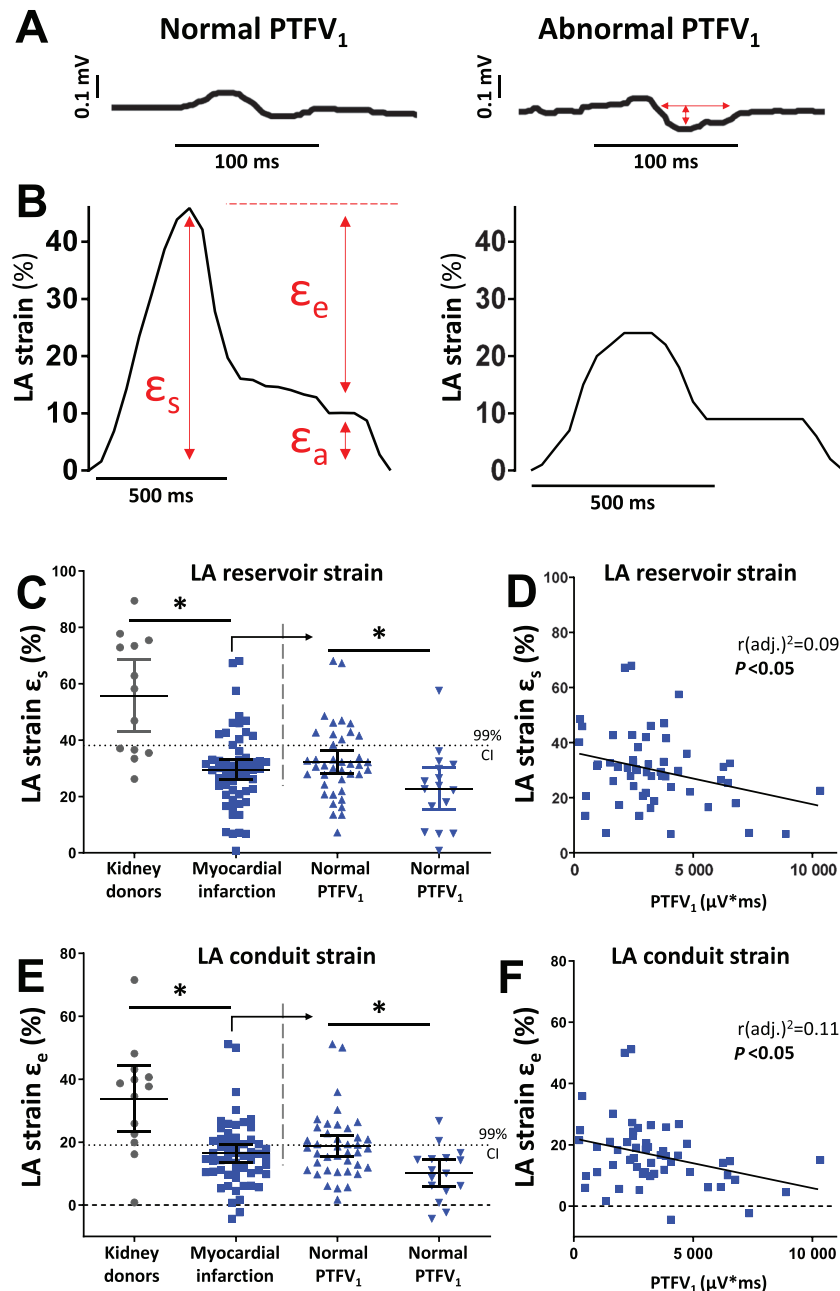
Atrial CMR strain measurements were performed as a sub-analysis of a prospective observational study in 56 patients with a first acute MI that was treated with percutaneous coronary intervention.[15] In order to report the range of physiological atrial strain, CMR acquisitions from 13 age-matched and gender-matched control individuals (potential living kidney donors)[16] were also analysed for atrial strain.

Atrial biopsies were collected from 32 patients who underwent elective coronary artery bypass grafting (CABG) in the prospective observational study CONSIDER-AF.[17]

Quantification of P-wave terminal force in lead V_1

P-wave terminal force was defined by the negative area of the P-wave in ECG lead V_1 ($PTFV_1$; *Figure 1A*). This area was quantified by multiplying duration and amplitude (ms*µV). A $PTFV_1$ of at least 4000 ms*µV (*Figure 1A*) was defined as abnormal, which is in accordance with previous studies.[18]

Figure 1 An abnormal PTFV$_1$ indicates reduced atrial function. (A) Tracing of an ECG and LA strain in a patient with normal and abnormal PTFV$_1$. Red arrows indicate measured values. PTFV$_1$ is calculated as the product of the duration and depth of the negative deflection of biphasic P-waves in the precordial ECG lead V$_1$. (B) CMR feature tracking tracings of global longitudinal strain of the LA are used to calculate atrial strain parameters for reservoir (εs) and conduit (εe) function. (C–F) Left atrial reservoir and conduit function for the kidney donors (n = 13, grey dots) and the myocardial infarction group (n = 56, blue squares); the dotted line represents the lower 99% CI of the kidney donors; the myocardial infarction group is further divided in normal and abnormal PTFV$_1$. (C) Left atrial reservoir function strain. (D) Correlation of PTFV$_1$ and LA reservoir strain. (E) Left atrial conduit function strain. (F) Correlation of PTFV$_1$ and LA conduit strain. CI, confidence interval; LA, left atrial; PTFV$_1$, P-wave terminal force V$_1$; ε, strain. *$P < 0.05$, Student's t-test. Exact values of means and P-values are listed in Supporting Information, *Table S2*.

Atrial function

To assess atrial function, CMR strain measurements were performed in patients with acute MI and in kidney donors.

Longitudinal atrial strain was computed as (L1 − L0)/L0, where L0 is the resting/reference length [left ventricular (LV) end-diastole] and L1 is the change of atrial myocardial length throughout the cardiac cycle. Atrial strain was

analysed for total strain (εs, corresponding to atrial reservoir function), passive strain (εe, corresponding to atrial conduit function), and active strain (εa, corresponding to atrial booster function; *Figure 1B*). Strain rate was calculated as change in ε over time: peak positive strain rate (corresponding to atrial reservoir function), peak early negative strain rate (corresponding to atrial conduit function), and peak late negative strain rate (corresponding to atrial booster function).

Atrial electrical remodelling

Electrical remodelling was analysed in right atrial appendage biopsies of patients undergoing elective CABG. CaMKII activity was measured by a specific assay [expressed in (normalized to CaMKII expression)] and was used as a surrogate for atrial electrical remodelling. Moreover, microdissected trabeculae were electrically field stimulated, and the severity of arrhythmias was classified.[12]

Atrial structural remodelling

Volumetric atrial parameters were assessed by CMR in patients with acute MI and by echocardiography in patients undergoing CABG. The presence of fibrosis was histologically measured in atrial biopsies of the latter population.

Statistics

All investigators were blinded to the clinical data during the conduction and analysis of all experiments. Continuous clinical data are presented as means ± standard deviation, and categorical variables as total number with relative proportion. Experimental data are reported as means ± standard error of the mean, based on a single value for each patient. For the assessment of baseline distribution, Student's *t*-test, Mann–Whitney test, and ANOVA for continuous variables and χ^2 or Fisher's exact test for discrete variables were used as appropriate. For univariate regression analysis of left atrial (LA) conduit strain and abnormal $PTFV_1$, based on clinical and pathophysiological considerations, we chose the factors age, gender, body mass index, renal function [estimated glomerular filtration rate (eGFR)], LV function (ejection fraction), N-terminal pro-brain natriuretic peptide (NT-pro-BNP), MI with ST elevation, maximal levels for creatine kinase (CK max), and systolic blood pressure as important clinical covariates. Based on these univariate regression analyses, the factors with a *P*-value equal to or less than 0.3 were included in a multivariate regression model [age, LV ejection fraction (LVEF), NT-pro-BNP, and eGFR]. For univariate regression analysis of CaMKII activity and $PTFV_1$, based on clinical and pathophysiological considerations, we included age, gender, body mass index, existing paroxysmal AF, existing heart failure, and renal function (eGFR). Based on these univariate regression analyses, the factors with a *P*-value equal to or less than 0.3 were included in a multivariate regression model (only $PTFV_1$ and age). We used IBM SPSS Statistics 25 for univariate or multivariate linear regression analyses. Statistical significance was defined by two-sided *P*-values below 0.05.

Results

Characteristics of patients with cardiac magnetic resonance imaging analyses

The baseline characteristics of kidney donors (*n* = 13) and patients after acute MI (*n* = 56) are listed in Supporting Information, *Table S1*. A total of 3/13 (23%) of the kidney donors had a $PTFV_1 \geq 4000$ ms*μV. *Table 1* presents the baseline characteristics of the MI patient cohort stratified into normal (*n* = 40) and abnormal $PTFV_1$ (*n* = 16). Patients with abnormal $PTFV_1$ were more likely to present with reduced LVEF and elevated NT-pro-BNP levels at discharge (*Table 1*).

Left atrial function is impaired in patients with abnormal P-wave terminal force in lead V_1

As there exist only sparse and incongruent data on normal atrial strain values, we measured LA strain in kidney donors. In accordance with impaired LA contractile function, LA reservoir, conduit, and booster function were all significantly depressed in patients after MI compared with kidney donors (*Figure 1C–F*, Supporting Information, *Figure S3*, and Supporting Information, *Table S2*).

Importantly, within the cohort presenting with MI, patients with an abnormal $PTFV_1$ showed significantly more impaired LA reservoir and conduit strain compared with patients with normal $PTFV_1$ (*Figure 1C + E*). In accordance, the magnitude of $PTFV_1$ and strain for LA reservoir and LA conduit function correlated significantly negative (*Figure 1D + F*). Strain rate is calculated as the change in strain over time, which means that reservoir strain rate has positive values whereas conduit and booster strain rates have negative values. Similarly, in patients with abnormal $PTFV_1$, LA reservoir strain rate was significantly lower, but LA conduit strain rate was significantly larger compared with MI patient with normal $PTFV_1$ (Supporting Information, *Figure S3*). There was also a significant correlation between the magnitude $PTFV_1$ and both the latter parameters (Supporting Information, *Figure S3*).

In contrast, there were no differences between groups for LA booster function, which describes the active atrial contraction (Supporting Information, *Table S2*).

Table 1 Baseline characteristics of patients with acute myocardial infarction

	Normal PTFV$_1$ ($n = 40$)	Abnormal PTFV$_1$ ($n = 16$)	P-value
PTFV$_1$ (ms*µV), mean ± SD	2353.5 ± 1072.2	6060.8 ± 1808.6	
Age (years), mean ± SD	53.9 ± 9.9	57.9 ± 9.6	0.173^T
Male, n (%)	34 (85.0%)	11 (68.7%)	0.263^F
Body mass index (kg/m^2), mean ± SD	28.5 ± 3.1	28.8 ± 4.0	0.771^T
Cardiovascular risk factors			
Arterial hypertension, n (%)	19 (47.5%)	9 (60%)	0.408^{Chi}
Diabetes mellitus, n (%)	6 (15%)	3 (20%)	0.705^F
Current or previous smoker, n (%)	30 (75%)	11 (73.5%)	0.741^F
Hypercholesterolaemia, n (%)	12 (30%)	5 (33.3%)	1.000^F
Heart and renal function			
STEMI, n (%)	3 (8.8%)	4 (33.3%)	0.094^F
CK max (U/L), mean ± SD	1993.5 ± 1393.2	2232.1 ± 1588.6	0.59^T
NT-pro-BNP at discharge, (pg/mL), mean ± SD	774.5 ± 835.6	2201.2 ± 1390.4	<0.001^T
LVEF (%), mean ± SD	48.7 ± 7.5	42.7 ± 11.8	**0.037^T**
LVEF classification			0.102^F
Normal EF ≥ 50%, n (%)	22 (55.0%)	6 (37.5%)	
Mildly reduced EF 40–49%, n (%)	15 (37.5%)	5 (31.3%)	
Moderately reduced EF 30–39%, n (%)	3 (7.5%)	4 (25.0%)	
Severely reduced EF ≤ 30%, n (%)	0 (0%)	1 (6.3%)	
LV mass index (g/m^2), mean ± SD	70.0 ± 12.6	80.8 ± 14.7	**0.006^T**
LV end-diastolic volume (mL), mean ± SD	155.8 ± 38.3	170.8 ± 38.1	0.161^T
LAVI (mL/m^2), mean ± SD	27.1 ± 12.8	33.2 ± 17.1	0.208^T
RV ejection fraction (%), mean ± SD	59.6 ± 8.2	57.7 ± 10.5	0.500^T
RV end-diastolic volume (mL), mean ± SD	134.4 ± 29.5	128.9 ± 28.5	0.525^T
RA volume index (mL/m^2), mean ± SD	13.5 ± 2.3	12.7 ± 2.4	0.287^T
Systolic blood pressure (mmHg), mean ± SD	127.4 ± 22.8	127.7 ± 17.7	0.970^T
eGFR (mL/min/1.73 m^2), mean ± SD	95.2 ± 16.5	83.6 ± 28.0	0.065^T
Medication			
ACE-inhibitor/AT1-blocker, n (%)	39 (97.5%)	16 (100%)	1.000^F
Beta-blocker, n (%)	39 (97.5%)	15 (93.7%)	0.494^F
Aldosterone receptor antagonists, n (%)	18 (45%)	11 (68.7%)	0.144^F
Loop diuretics, n (%)	16 (40%)	9 (56.2%)	0.269^{Chi}

ACE, angiotensin-converting enzyme; BNP, brain natriuretic peptide; Chi, χ^2 test; CK, creatine kinase; EF, ejection fraction; eGFR, estimated glomerular filtration rate; F, Fisher's exact test; LA, left atrial; LV, left ventricular; NT-pro-BNP, N-terminal pro-brain natriuretic peptide; PTFV$_1$, P-wave terminal force in lead V$_1$; RA, right atrial; RV, right ventricular; SD, standard deviation; STEMI, ST-elevation myocardial infarction; T, Student's t-test.
Clinical characteristics of patients with acute myocardial infarction and cardiac magnetic resonance imaging cine data. Bold values mean statistical significance calculated by two-sided Student's t-test, χ^2 test, or Fisher's exact test, as appropriate.

Table 2 Abnormal PTFV$_1$ predicts independently pathological atrial function

$n = 56$ LA conduit strain (%)	Univariate linear regression analysis		Multivariate linear regression analysis Adj. $r^2 = 0.402$; $P < 0.001$	
	B (95% CI)	P-value	B (95% CI)	P-value
Abnormal PTFV$_1$ (≥4000 µV*ms)	−8.703 (−14.521; −2.885)	**0.004**	−7.336 (−13.577; −1.095)	**0.022**
Age (years)	−0.426 (−0.691; −0.160)	**0.002**	−0.416 (−0.683; −0.149)	**0.003**
LVEF (%)	0.548 (0.280; 0.815)	**<0.001**	0.419 (1.121; 0.717)	**0.007**
NT-pro-BNP (pg/mL)	−0.002 (−0.004; 0.000)	0.096	0.002 (−0.001; 0.004)	0.171
CK max (U/L)	−0.002 (−0.004; 0.000)	0.121	−0.001 (−0.003; 0.001)	0.478
STEMI	−1.158 (−13.077; 10.762)	0.845		
Male	−0.893 (−8.034; 6.248)	0.803		
Body mass index (kg/m^2)	−0.186 (−1.059; 0.687)	0.671		
systolic blood pressure (mmHg)	0.025 (−0.111; 0.160)	0.717		

CI, confidence interval; CK, creatine kinase; LA, left atrial; LVEF, left ventricular ejection fraction; NT-pro-BNP, N-terminal pro-brain natriuretic peptide; PTFV$_1$, P-wave terminal force in lead V$_1$; STEMI, ST-elevation myocardial infarction.
Linear regression analyses between an abnormal PTFV$_1$ and left atrial conduit strain in patients with acute myocardial infarction. Bold font is used to highlight statistically significant P-values < 0.05.

Diagnostic value of P-wave terminal force in lead V$_1$ for reduced atrial function

Baseline characteristics (*Table 1*) suggested that patients with heart failure were more likely to show abnormal PTFV$_1$ and ventricular contractile dysfunction that can substantially impact atrial strain. Therefore, we have performed univariate and multivariate linear regression for reduced LA conduit function and PTFV$_1$ and important clinical confounders (*Table 2*). We have chosen LA εe among the strain

parameters, because it showed the strongest relationship with PTFV$_1$ (largest r^2; *Figure 1F* and Supporting Information, *Figure S3C*).

Univariate regression analysis revealed that besides PTFV$_1$, the parameters age and LVEF significantly correlated with LA εe. Interestingly, incorporating age, eGFR, LVEF, and NT-pro-BNP in a multivariate model, abnormal PTFV$_1$ was independently associated with impaired atrial strain, suggesting that PTFV$_1$ might be a valuable independent marker for atrial function.

To investigate which clinical parameters influence the magnitude of PTFV$_1$, we performed univariate regression analyses using parameters for cardiac function, cardiac injury, biometric data, and co-morbidities (Supporting Information, *Table S3*). Besides LA strain, there was a significant association with NT-pro-BNP levels at discharge, and a strong trend for magnitude of LVEF confirming the importance of LV function for atrial electrical remodelling and corroborating the consideration of LV dysfunction as an important confounder.

Baseline characteristics of patients undergoing elective coronary artery bypass grafting

To investigate possible pathomechanisms for the development of a PTFV$_1$-detected atrial cardiomyopathy, we have analysed atrial biopsies of 32 patients undergoing elective CABG, stratified by a normal (*n* = 17) and an abnormal PTFV$_1$ (*n* = 15, Supporting Information, *Figure S2* and Supporting Information, *Table S4*). There were no differences between both groups regarding important clinical characteristics.

Abnormal P-wave terminal force in lead V$_1$ predicts atrial electrical remodelling

Because CaMKII is a key regulator of cardiac ion homeostasis and may underlie atrial electrical remodelling,[14] we measured CaMKII activity using a highly specific HDAC4 pull-down assay. Intriguingly, CaMKII activity was significantly increased in patients with an abnormal PTFV$_1$ (0.87 ± 0.17 vs. 1.46 ± 0.15, *P* = 0.047; *Figure 2B*). There was also a significant positive correlation of CaMKII activity and the magnitude of PTFV$_1$ (r^2 = 0.21, *n* = 20, *P* = 0.04; *Figure 2C*).

Because CaMKII has been shown to foster the occurrence of pro-arrhythmic events,[13,14] we measured premature atrial contractions (PACs) in human atrial trabeculae, which are a surrogate for multicellular arrhythmias.[12] Interestingly, trabeculae from patients with an abnormal PTFV$_1$ exhibited a significantly increased severity of PACs resulting in a significant positive correlation between the magnitude of PTFV$_1$ and PACs severity (r^2 = 0.19, *n* = 30, *P* = 0.02; *Figure 2D–F*). Intriguingly, specific CaMKII inhibition with KN93 significantly reduced the incidence and severity of atrial pro-arrhythmic

activity in patients with abnormal PTFV$_1$. PAC severity decreased from 3.55 ± 0.47 (*n* = 14) to 1.18 ± 0.63 (*n* = 11, *P* = 0.008), a level that was comparable with patients with a normal PTFV$_1$ (1.25 ± 0.58, *n* = 12; *Figure 2E*). CaMKII inhibition with KN93 also abolished the positive correlation between magnitude of PTFV$_1$ and PAC severity (*Figure 2F*). In contrast, the inactive analogue KN92, which does not inhibit CaMKII, did not reduce the severity of PACs, suggesting that atrial pro-arrhythmic activity in patients with an abnormal PTFV$_1$ was due to CaMKII-dependent signalling (*Figure 2E*).

As presented in Supporting Information, *Table S4*, the investigated patients had multiple co-morbidities that could substantially confound our observations. To control for this potential limitation, we conducted univariate and multivariate linear regression analyses, accounting for age, sex, body mass index, existing paroxysmal AF, existing heart failure, and glomerular filtration rate. Most importantly, the correlations of abnormal PTFV$_1$ with CaMKII activity (*Table 3*) or the severity of trabecular arrhythmias (Supporting Information, *Table S5*) were independent from potential confounders, suggesting that the magnitude of PTFV$_1$ may be a good clinical measure for abnormal atrial CaMKII signalling.

Abnormal P-wave terminal force in lead V$_1$ is not indicative of structural remodelling

In contrast to parameters of atrial function and electrical remodelling, CMR parameters of structural remodelling (e.g. LA volume index and LA fractional area change) showed no significant correlation with PTFV$_1$ in patients after acute MI (Supporting Information, *Table S3*).

Consistent with this, echocardiographic measurements in patients undergoing elective CABG showed that an abnormal PTFV$_1$ was not associated with atrial size (Supporting Information, *Table S4*). We further analysed structural remodelling by histological measurement of fibrosis in atrial biopsies of patients undergoing CABG. Surprisingly, the area of fibrotic tissue was significantly reduced in patients with an abnormal PTFV$_1$ (12.32 ± 1.63%, *n* = 13 vs. 20.50 ± 2.09%, *n* = 17; *Figure 3B*). In addition, there was a significant negative correlation between the magnitude of PTFV$_1$ and the severity of atrial fibrosis (r^2 = 0.17, *n* = 30, *P* = 0.02; *Figure 3B*). After accounting for potential clinical confounders, multivariable regression analysis revealed a significant and independent inverse association between abnormal PTFV$_1$ and severity of atrial fibrosis (Supporting Information, *Table S6*). Accordingly, we found a significant negative correlation between the area of fibrotic tissue and CaMKII activity (r^2 = 0.36, *n* = 19, *P* = 0.006; *Figure 3C*). Interestingly, only the amplitude, but not the duration of the terminal force, correlated significantly negative with the area of fibrotic tissue (r^2 = 0.21, *n* = 30, *P* = 0.01; Supporting Information, *Figure S4*).

Figure 2 Electrical remodelling in patients with an abnormal PTFV$_1$. (A) Original Western blot for the analysis of CaMKII activity, assessed by a specific HDAC4 (Histone Deacetylase 4) pull-down assay in atrial homogenates of CABG patients with a normal and an abnormal PTFV$_1$. (B) Densiometric analysis revealed a significantly increased CaMKII activity (CaMKII-HDAC4 binding normalized to CaMKII expression) in patients with an abnormal PTFV$_1$ (n = 9 vs. 11). (C) Moreover, linear regression analysis showed a significant positive correlation between PTFV$_1$ and CaMKII activity (n = 20). (D) Representative original recordings of electrically stimulated trabeculae (stimulated contractions indicated by red vertical lines) for a patient with a normal PTFV$_1$ and a patient with an abnormal PTFV$_1$ before and after wash-in of the CaMKII inhibitor KN93. (E) Intriguingly, the mean severity of premature atrial contractions (PACs) was significantly increased in patients with an abnormal PTFV$_1$ (n = 14 vs. 16) but could be significantly reduced by CaMKII inhibition with KN93 (n = 11). Moreover, the inactive analogue KN92 did not show any anti-arrhythmic effect. (F) Additionally, we observed a significant positive correlation between PTFV$_1$ and the severity of PACs (n = 30). Interestingly, this correlation was completely abolished upon CaMKII inhibition with KN93 (n = 23). *$P < 0.05$ vs. normal PTFV$_1$, $^\#P < 0.05$ vs. ISO + Ca, Mann–Whitney test, two-way ANOVA post hoc corrected by Holm–Sidak, and linear regression analysis, as appropriate.
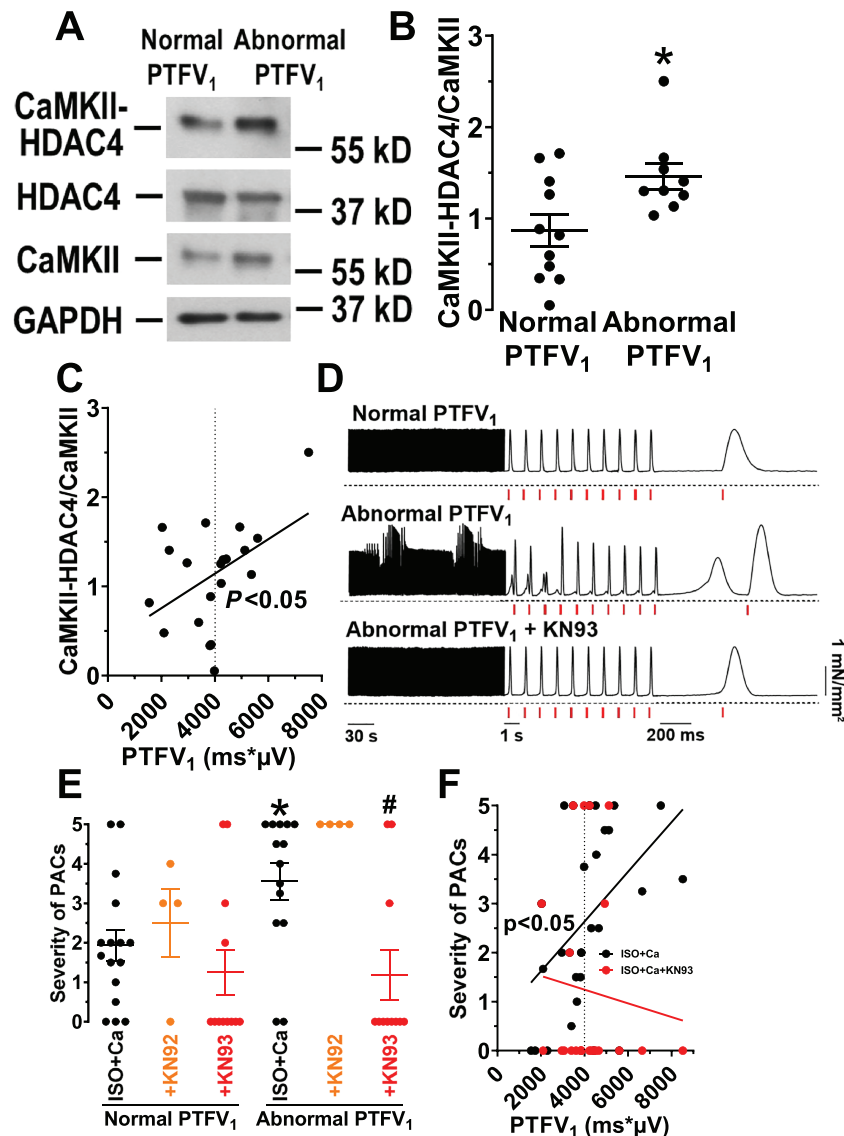
In contrast to the amplitude, the duration of intra-atrial and atrioventricular conduction appears to depend on the magnitude of fibrotic tissue. Interestingly, the PQ interval as a measure of atrial conduction correlated significantly positive with the area of fibrotic tissue (r^2 = 0.14, n = 30, P = 0.04; *Figure 3D*).

Discussion

Atrial functional, electrical, and structural abnormalities such as reduced focal contractility, intermittent non-recordable arrhythmias, or fibrosis have been suggested to result in a pro-thrombotic and pro-arrhythmogenic atrial disease called

Table 3 Abnormal PTFV$_1$ is an independent predictor for CaMKII activity

n = 20	Univariate linear regression analysis		Multivariate linear regression analysis Adj. r^2 = 0.255; P = 0.032	
CaMKII activity (normalized to CaMKII expression)	B (95% CI)	P-value	B (95% CI)	P-value
Abnormal PTFV$_1$ (≥4000 ms*μV)	0.591 (0.106; 1.077)	**0.020**	0.542 (0.057; 1.027)	**0.031**
Age (years)	−0.022 (−0.053; 0.008)	0.143	−0.017 (−0.045; 0.011)	0.210
Male	0.032 (−0.620; 0.683)	0.920		
Body mass index (kg/m^2)	0.012 (−0.058; 0.082)	0.729		
Paroxysmal atrial fibrillation	0.205 (−0.579; 0.988)	0.590		
Existing heart failure	0.035 (−0.540; 0.611)	0.899		
eGFR (mL/min/1.73 m^2)	−0.001 (−0.012; 0.009)	0.781		

CaMKII, Ca/calmodulin-dependent protein kinase II; CI, confidence interval; eGFR, estimated glomerular filtration rate; PTFV$_1$, P-wave terminal force in lead V$_1$.
Linear regression analyses between an abnormal PTFV$_1$ and CaMKII activity in right atrial appendage biopsies of patients undergoing coronary artery bypass grafting. Bold font is used to highlight statistically significant P-values < 0.05.

atrial cardiomyopathy.[2,6] However, urgently needed clinical markers that identify patients with atrial cardiomyopathy that are at increased risk for AF or stroke are currently missing. We show here that the presence of an abnormal PTFV$_1$ significantly correlates with reduced atrial strain and increased CaMKII-dependent atrial pro-arrhythmic activity in contrast to structural atrial remodelling and independent from important clinical confounders (graphical abstract).

P-wave terminal force in lead V$_1$ is a marker for reduced atrial function

A large body of evidence has linked impaired atrial strain to reduced cardiovascular outcome.[4,19] Several studies have shown good feasibility and reproducibility of feature tracking assessment of LA strain parameters in healthy and in acutely ill patients.[20–22] Strain assessment of the LA in echocardiography and CMR is fairly well established but not yet routinely applied.[23] Reduced atrial strain is a sensitive marker of atrial dysfunction, and its evaluation improves the accuracy of risk stratification in multiple cardiac pathologies. LA strain measurement provides risk assessment for stroke in patients with AF in addition to the CHA$_2$DS$_2$-VASc score.[24]

Accordingly, our data show that LA strain is reduced after acute MI compared with kidney donor subjects (*Figure 1* and Supporting Information, *Figure S3*). As there are sparse and incongruent data on normal LA strain values,[23] we included the strain data of the kidney donors to define pathological strain values as values below the lower 99% confidence interval (*Figure 1C + E* and Supporting Information, *Figure S3B + C*). However, despite the useful additional information, CMR is not generally available in most settings of cardiovascular care.

In that respect, the presence of an abnormal PTFV$_1$ derived from ubiquitously available ECG analysis has recently been associated with atrial remodelling.[2,6] Moreover, an abnormal PTFV$_1$ predicts an increased risk for AF and was independently associated with the incidence for cryptogenic or cardioembolic stroke.[6,9,10] Additionally, an abnormal PTFV$_1$ has

been shown to be an independent predictor for hospitalization and for increased mortality due to cardiovascular disease.[5]

However, the mechanisms that result in abnormal PTFV$_1$ are poorly understood. It was hypothesized that altered haemodynamics may cause subtle deformation of the LA, which then changes the electrical vector in the ECG and elevates PTFV$_1$ amplitude.[25]

We show here that an abnormal PTFV$_1$ was independently associated with impaired LA reservoir and conduit function (*Figure 1*). It appears reasonable to assume that PTFV$_1$ may be influenced by a multitude of factors that alter not only atrial function but also ventricular function and haemodynamics. Importantly, we show here by linear regression that impaired LA conduit strain (besides NT-pro-BNP) was the strongest predictor for abnormal PTFV$_1$, but LVEF despite showing a clear trend did not reach statistical significance (Supporting Information, *Table S3*).

Electrical remodelling and P-wave terminal force in lead V$_1$

Because atrial contractile function and atrial arrhythmogenesis have both been shown to be regulated by CaMKII, and increased expression and activity of CaMKII are hallmarks of contractile dysfunction and arrhythmias in patients with AF[26] and heart failure,[14] we measured atrial CaMKII activity in a high-risk cohort of cardiovascular patients undergoing CABG. We show here that increased atrial CaMKII activity was significantly correlated with abnormal PTFV$_1$ (*Figure 2A–C*). Many previous studies investigating human myocardium were limited regarding scarce data about inclusion criteria, and patient characteristics with important co-morbidities were often not available. The information about co-morbidities, for example, heart failure or AF, however, are of utmost importance as these are tightly linked with atrial cardiomyopathy. Both heart failure and AF have already been shown to be associated with an increased CaMKII-dependent pro-arrhythmic activity.[12–14] To account

Figure 3 Atrial fibrosis is decreased in patients with an abnormal PTFV$_1$. (A) Original micrographs showing myocardial fibrosis (Masson's trichrome staining) in trabeculae of CABG patients with a normal and an abnormal PTFV$_1$ (400× magnification). (B) Interestingly, the area of myocardial fibrosis (%) was significantly reduced in patients with an abnormal PTFV$_1$ (n = 13 vs. 17), leading to a significant negative correlation with the PTFV$_1$ (n = 30). (C) Moreover, we also found the area of fibrosis correlating significantly negative with the CaMKII activity, indicating that active CaMKII can only be found in cardiomyocytes and not in fibrotic tissue (n = 19). (D) Additionally, we found the area of fibrosis correlating significantly positive with a slowed atrial conduction velocity (i.e. PQ time, n = 30). *P < 0.05, Mann–Whitney test and linear regression analysis, as appropriate.
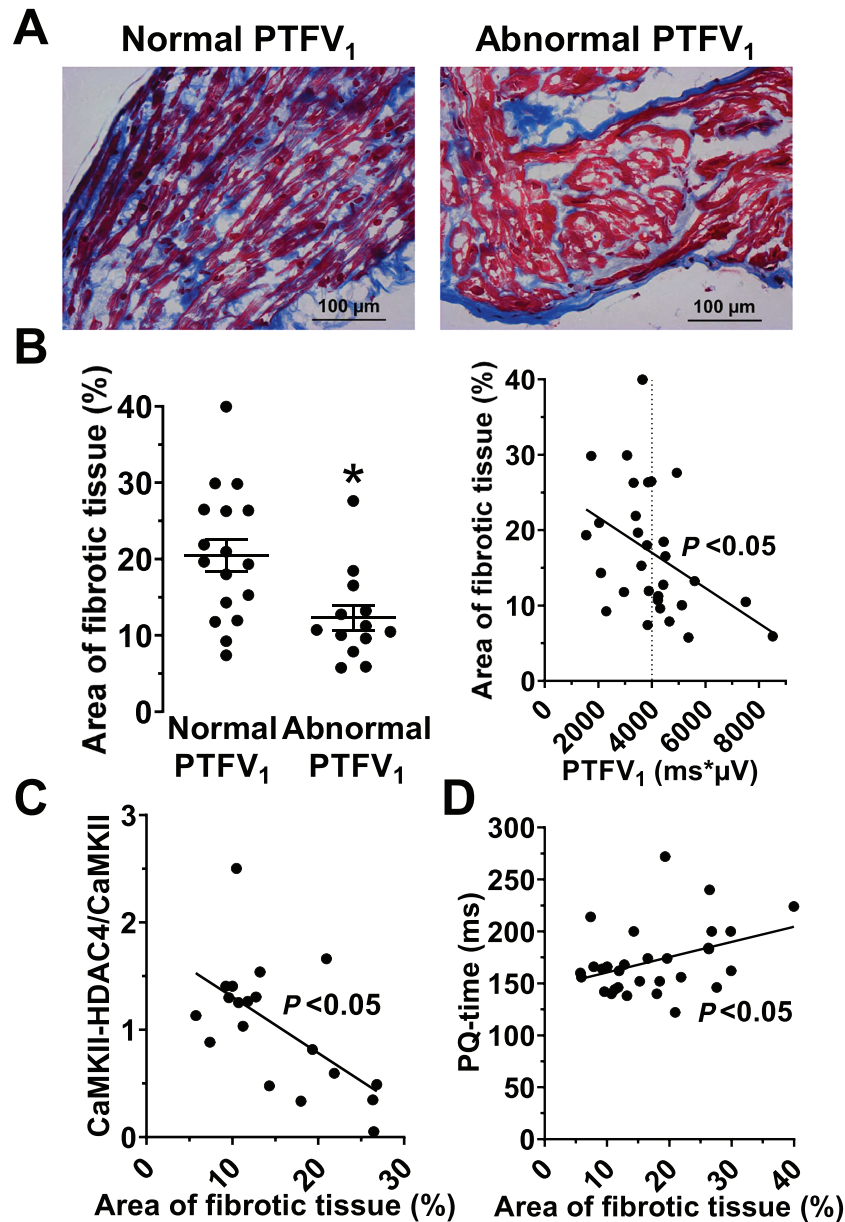
for this, we conducted multivariate linear regression analyses using the important clinical covariates age, gender, body mass index, existing paroxysmal AF, existing heart failure, and glomerular filtration rate. We show here that the increase of CaMKII activity in patients with an abnormal PTFV$_1$ was independent from important co-morbidities (*Table 3*).

Interestingly, it has been shown previously that increased CaMKII activity results in an impairment of cardiomyocyte Na and Ca homeostasis leading to increased cardiomyocyte triggered activity due to the promotion of early and delayed afterdepolarizations.[2,12–14]

To better understand the relationship between an abnormal PTFV$_1$, enhanced CaMKII signalling, and atrial arrhythmias, we measured atrial pro-arrhythmic activity. We here show that the magnitude of PTFV$_1$ correlated significantly and independently positive with the severity of trabecular

arrhythmias. Moreover, selective CaMKII inhibition with KN93 significantly reduced the severity of trabecular arrhythmias, indicating the pivotal role of CaMKII for arrhythmogenesis in patients with an abnormal $PTFV_1$ (*Figure 2E*).

An abnormal P-wave terminal force in lead V_1 does not indicate structural remodelling

Besides disturbed cardiomyocyte Na and Ca homeostasis, structural remodelling (e.g. fibrosis) is another feature of atrial cardiomyopathy.[2] In the present study, linear regression models revealed no significant correlation between $PTFV_1$ and the volumetric LA parameters area, fractional area change, and volume index (Supporting Information, *Table S3* for CMR data and Supporting Information, *Table S4* for echocardiographic data), suggesting that $PTFV_1$ does not indicate LA enlargement. This is in accordance with a study in healthy athletes also showing no significant differences for median LA diameter and median LA end-systolic volume in individuals with an abnormal $PTFV_1$.[27]

Interestingly, atrial structural remodelling in patients with atrial cardiomyopathy is frequently accompanied by increased fibrosis development. Moreover, long-term tachycardia or AF itself have been shown to induce atrial fibrosis that is essential for the progression to permanent AF.[2] Therefore, we investigated the magnitude of fibrosis in atrial biopsies of high-risk cardiovascular patients undergoing CABG. Surprisingly, we observed that patients with abnormal $PTFV_1$ showed significantly less atrial fibrosis and that this decrease was independent from important clinical covariates (*Figure 3* and Supporting Information, *Table S6*). At first glance, this result may appear counterintuitive, especially, because a previous study suggested that ventricular fibrosis may be increased in patients with abnormal $PTFV_1$.[28] There are several explanations for this discrepancy. We have confirmed atrial fibrosis by histochemical analysis using Masson's trichrome stain. Tiffany Win and colleagues have used surrogate markers measured by magnetic resonance imaging (late gadolinium enhancement) and only for ventricular myocardium, which may be prone to misinterpretation.[28] Another explanation is based on the fact that only vital cardiomyocytes, but not fibrotic tissue, are electrically active. Thus, increased fibrosis —that is accompanied by a reduced number of vital cardiomyocytes—may be less able to generate terminal force amplitude. In our analysis, the magnitude of fibrosis was associated with reduced terminal force amplitude, which supports this hypothesis (Supporting Information, *Figure S4*). One could argue that, on the other hand, increased fibrosis may have slowed atrial conduction resulting in increased terminal force duration. We show here, however, that terminal force duration was not correlated with atrial fibrosis (Supporting Information, *Figure S4*), excluding the possibility that slowed

atrial conduction contributed to the magnitude of $PTFV_1$. In accordance, the magnitude of fibrosis was also negatively correlated to CaMKII activity (*Figure 3C*), and the latter is almost exclusively expressed in cardiomyocytes and not in fibrotic tissue. This suggests that the magnitude of $PTFV_1$ may be a good marker for increased electrical remodelling in vital cardiomyocytes possibly at earlier stages of atrial cardiomyopathy when structural remodelling is not yet prevailing. Interestingly, consistent with the concept of fibrosis slowing conduction, we observed a significant positive correlation between the duration of the PQ interval and the magnitude of fibrosis (*Figure 3D*). A possible explanation for the different effect of fibrosis on intra-atrial vs. atrioventricular conduction may be the very high atrial conduction reserve.[29] The conduction velocity in the AV node, on the other hand, may be much more sensitive to fibrosis.

Limitations

A potential limitation is that LA functional, electrical, and structural remodelling were measured in two distinct populations, which might confound our observations. Unfortunately, as standardized CMR acquisition and human atrial biopsies are each rare and difficult to obtain, there were no patients with both. At least, observations for $PTFV_1$ and structural remodelling (volumetric CMR and echocardiographic measurements) were consistent in both populations.

Another potential limitation may be that the diagnosis of heart failure, which is frequently associated to CaMKII-dependent pro-arrhythmic activity and remodelling, was no prespecified inclusion criterium. However, substantial fractions of heart failure patients were present in the MI and CABG cohorts, and multivariate regression analyses revealed that LV dysfunction did not affect atrial strain or atrial structural remodelling in our cohorts. Nevertheless, we cannot exclude that in a more selected heart failure cohort, LV dysfunction would also result in impaired atrial strain and structural remodelling.

We used kidney donors as a control group who underwent a thorough medical evaluation to be eligible for kidney donation. Strain values of these presumed healthy individuals may therefore provide insight into normal strain ranges. However, the small number of 13 patients in this group may not allow broad generalizability of these results.

P-wave terminal force in lead V_1 may be a novel clinical marker for early atrial cardiomyopathy

Our data suggest $PTFV_1$ to be an independent marker for early atrial cardiomyopathy, characterized by functional and electrical, but not yet irreversible structural remodelling, which may have several diagnostic and therapeutic

implications. Firstly, we found PTFV$_1$ predicting atrial pro-arrhythmic activity. Paroxysmal AF may only be detected by very long-term ECG monitoring and is often clinically inapparent, yet possibly resulting in severe sequelae like cardio-embolic stroke.[1,6] Atrial cardiomyopathy may result in a pro-thrombotic environment in absence of AF.[2,6] Therefore, PTFV$_1$ may be a selection criterion for extended long-term ECG monitoring.

Moreover, clinical evidence suggests that the extent of atrial fibrosis is a critical determinant for a successful atrial ablation procedure.[1] However, measurement of atrial fibrosis by CMR is not an easy task and far away from clinical routine. We found PTFV$_1$ as a biomarker for reduced atrial fibrosis, which may help to identify the patients with the highest success rate for atrial ablation procedures.

Finally, an abnormal PTFV$_1$ may even serve as a novel and readily available clinical marker for enhanced CaMKII-dependent pro-arrhythmic activity. This is of special relevance because several CaMKII inhibitors are currently under preclinical consideration.[13] Thus, an abnormal PTFV$_1$ may guide future application of CaMKII inhibitors for the treatment of atrial cardiomyopathy.

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Conflict of interest

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Study flowchart for patients with acute MI.
Figure S2. Study flowchart for patients undergoing elective CABG.
Figure S3. Strain rate values for kidney donors and patients with acute MI.
Figure S4. Trabecular fibrosis alters electrical conduction.
Table S1: Baseline characteristics of all patients with CMR data.
Table S2: Atrial strain from CMR.
Table S3: Univariate linear regression for the magnitude of PTFV$_1$.
Table S4: Baseline characteristics of patients undergoing CABG.
Table S5: Abnormal PTFV$_1$ as an independent predictor for the severity of trabecular arrhythmias.
Table S6: Abnormal PTFV$_1$ is negatively and independently associated with atrial fibrosis.

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