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Prolongation of PR interval is associated with endothelial dysfunction and activation of vascular repair in high-risk cardiovascular patients

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Abstract
Purpose Epidemiological studies showed that PR prolongation is associated with increased risk of adverse cardiovascular outcomes. We investigated the relations of PR interval with indices of vascular function and endothelial repair as the underlying mechanisms.
Methods The study comprised 348 high-risk patients with prior coronary artery disease, ischemic stroke, and/or diabetes mellitus recruited from medical outpatient clinics and 150 healthy subjects without such a history. PR interval was considered prolonged if >200 ms, as determined from resting 12-lead electrocardiogram. Vascular function was assessed by brachial flow-meditated dilatation (FMD) using high-resolution ultrasound. Circulating $CD133^+/KDR^+$ endothelial progenitor cell (EPC) levels were measured by flow cytometry.
Results Among healthy subjects, PR interval was inversely associated with FMD ($R=-0.20$, $P=0.015$), but not with the level of circulating $CD133^+/KDR^+$ EPC ($R=0.05$, $P=0.58$). Among high-risk cardiovascular patients, PR prolongation >200 ms was more common compared with healthy subjects (45/348 (13 %) versus 4/150 (3 %), $P<0.001$). PR interval was associated inversely with FMD ($R=-0.14$, $P=0.01$) and positively with circulating $CD133^+/KDR^+$ EPC level ($R=+0.14$, $P=0.009$). Circulating $CD133^+/KDR^+$ EPC level was significantly increased in patients with PR prolongation >200 ms (0.87 ± 0.37 versus 0.68 ± 0.42 (log, $\times10^{-3}$/ml), $P=0.005$). Adjusted for potential confounders, increased PR interval remained independently associated with increased $CD133^+/KDR^+$ EPC by +0.002 (95 % confidence interval (CI) 0.000 to 0.004 (log, $\times10^{-3}$/ml), $P=0.011$) and depressed FMD ($B=-0.014$ %, 95 % CI −0.027 to −0.002, $P=0.026$).
Conclusions PR prolongation is associated with endothelial dysfunction and evidence of endothelial repair activation in patients with high cardiovascular risk.

Keywords PR prolongation · Endothelial dysfunction · Endothelial progenitor cells · Adverse cardiovascular outcomes

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1 Introduction

First-degree heart block, defined as prolongation of the PR interval >200 ms on electrocardiogram (ECG) (AHA/ACC guidelines) [1], is a commonly encountered clinical finding [2] and occurs in the population setting with a prevalence of 0.7–2 % in the young and up to 14 % in the elderly in their 80s [3]. Despite the traditional belief that PR interval prolongation is a usually benign conduction disorder, two large community studies (the Framingham Heart Study and the Atherosclerosis Risk in Community Study (ARIC)) showed that in otherwise healthy persons, PR interval prolongation is associated with increased risk of incident atrial fibrillation

(by onefold), pacemaker implantation (by twofold), and all-cause mortality (by 40 %) [4, 5]. In the Heart and Soul Study [6], patients with stable coronary artery disease and prolongation of PR interval had increased risks of heart failure hospitalization and cardiovascular death. However, the mechanisms underlying these adverse outcomes in subjects with PR interval prolongation are unclear.

Our recent exploratory study in healthy subjects without prior atherosclerotic cardiovascular disease (CVD) showed that PR interval prolongation is associated with endothelial dysfunction and increased arterial stiffness [7]. We suggested that PR interval prolongation may be a marker of subclinical CVD, mediated through endothelial dysfunction. However, in patients with established CVD, such a relationship is unknown. Given that impaired vascular function is independently predictive of recurrent cardiovascular events and that absolute risk of future events in patients with established CVD is substantially higher [8], identifying and understanding adverse vascular changes in these highly susceptible subjects have important clinical and secondary preventive implications.

In this study, we hypothesized that PR interval prolongation leads to adverse subclinical damage to the cardiovascular system in high-risk CVD patients, as in healthy subjects, and that such subclinical vascular damage can be gauged by surrogate indices of endothelial dysfunction and vascular repair. The purpose of this study was to explore the associations of PR interval with markers of vascular function and repair activation in a group of high-risk CVD patients.

2 Methods

2.1 Patients

The study comprised a total of 348 consecutive patients with documented coronary artery disease, atherothrombotic stroke, or diabetes mellitus who are at high-risk of future cardiovascular events, recruited from outpatient clinics. Patients with the following conditions were excluded: recent myocardial infarction, unstable angina, coronary revascularization, and stroke or acute heart failure within the past 6 months, dilated cardiomyopathy, significant valvular heart disease, chronic atrial fibrillation, cardioembolic stroke, New York Heart Association class III or IV heart failure, significant renal impairment with creatinine >220 mmol/l, liver failure, and clinical/biochemical evidence of concomitant inflammatory disease. All participants had a stable diet pattern and cardiovascular medications for at least 3 months prior to the date of recruitment.

For comparison, we further included a total of 150 healthy subjects who were randomly recruited from a community-based health screening program within the Hong Kong Island West Cluster network of the Hong Kong Hospital Authority, which is further built on the initial exploratory study of 88 healthy subjects [7] with additional measurement of vascular repair markers. Subjects with a history of any of the following conditions were excluded: coronary artery disease, atherothrombotic/hemorrhagic stroke, peripheral vascular disease, diabetes mellitus, heart failure, significant valvular heart disease, chronic atrial fibrillation, and significant liver/renal impairment.

All patients gave written informed consent to the study. The study was in accordance with the Declaration of Helsinki and approved by the institutional review board (Hong Kong West Cluster/The University of Hong Kong) of the University of Hong Kong.

2.2 Demographic, clinical, and laboratory evaluations

Baseline demographic data, cardiovascular risk factors, and cardiovascular medications were documented. Hypertension was defined as either resting systolic or diastolic blood pressure ≥140/90 mmHg at two different clinic visits or on medications. Diabetes mellitus was defined as serum fasting glucose ≥7.0 mmol/l (≥126 mg/dl) or on medications. Hypercholesterolemia was defined as a fasting total plasma cholesterol level ≥4.9 mmol/l (≥190 mg/dl) or on cholesterol-lowering medications. Smoking status was recorded as either past smoker, current smoker, or non-smoker. Family history of coronary artery disease was considered positive in first-degree relatives with diseases diagnosed at an age younger than 55 years. Long-term physical activity level was defined as nil, regular, or irregular exercise. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters using measurements during the visit. A standard 12-lead ECG was performed on all subjects after resting in a supine position for 5 min. PR interval was measured by computerized analysis of the ECG and then verified blindly using manual method. All complete 12-lead ECG tracings were studied manually at the usual size by an internal medicine physician blinded to the history of the patients for the presence of atrial fibrillation, Morbitz type I or II block, or third-degree heart block, as classified by the Minnesota criteria. None of these were identified in the patient sample. Any uncertainties or discrepancies were resolved through discussion and agreement with a senior consultant cardiologist. Fasting (12 h) blood samples were collected for analysis of serum low-density lipoprotein cholesterol (LDL-C), triglycerides, high-density lipoprotein cholesterol (HDL-C), and glucose.

2.3 Vascular ultrasound examination

Vascular ultrasound was performed with a high-resolution ultrasound system (Agilent Sonos 5500, Philips, USA)

using a 7.5-MHz linear array transducer by an experienced operator. All the scanned images were stored digitally and analyzed offline without knowledge of the subjects. Patients were studied in the fasting state. To avoid any systematic differences in diurnal variation of vascular reactivity, all studies were performed in the morning (time range, 0900–1200 hours). All vasoactive medications, cigarette smoking, caffeine drink, and alcohol consumption were withheld for at least 12 h before the assessment. As previously described [7, 9, 10], longitudinal scans of the brachial artery were obtained at rest, and then flow-mediated dilatation (FMD) was induced by inflation of a pneumatic tourniquet placed on the forearm to a pressure of 250 mmHg for 5 min. The cuff was then released, and serial imaging of the brachial artery was recorded for 5 min. The brachial artery was allowed to return to baseline. FMD was defined as the percentage change in brachial artery diameter by 1 min after cuff deflation from that on the baseline scan. Interobserver variability testing for FMD measurement revealed an interclass correlation coefficient (two-way mixed, random effect model, absolute agreement) of 0.83 (95 % confidence interval (CI) 0.22–0.97, P=0.012), with a mean absolute difference of 0.6±0.8 %.

2.4 Flow cytometry

Fluorescence-activated cell analysis was performed to determine the number of $CD133^{+}KDR^{+}$ endothelial progenitor cells (EPCs) [11, 12]. Blood was aliquoted into four portions for preservation under −70 °C before the flow cytometry. Briefly, 100 μl of peripheral blood was incubated with a fluorescein isothiocyanate (FITC)-conjugated CD133 antibody (Beckman Coulter, Fullerton, CA, USA). FITC-labeled anti-human CD45 antibody was used for differential gating during flow analysis. FITC-labeled IgG1a (Beckman Coulter) and phycoerythrin-labeled IgG2b (Becton Dickinson, Franklin Lakes, NJ, USA) served as the isotypic control for color compensation. Analysis was performed with an automated fluorescence-activated cell counter (Elite, Beckman Coulter) in which 1,000,000 events were counted. The absolute cell counts of all the measured components per 1,000,000 events in the lymphocyte gate were calculated. Circulating EPC count was log-transformed due to right-skewed distribution ($\times 10^{-3}$/ml).

2.5 Statistical analysis

Continuous variables were expressed as mean±1 standard deviation (SD). Statistical comparisons were performed using Student's t test or Fisher's exact test, as appropriate. Conventional normal range of the PR interval was adopted as <200 ms. Absolute changes and 95 % CI of FMD and $CD133^{+}KDR^{+}$ EPCs were calculated by univariable and multivariable linear regression analysis. The crude model included only PR interval as the explanatory variable. Multivariable analyses were performed with a forward stepwise regression model in which each potentially confounding variable with a P value ≤0.25 (based on the univariable analysis) was entered into the model. Analysis was further repeated and compared using a fully adjusted model in which all variables defined a priori based on the subject matter of relevance were entered. Considered variables include age, sex, smoking history, presence of diabetes mellitus, body mass index, physical activity, systolic and diastolic blood pressure, resting heart rate, LDL-C, HDL-C, triglycerides, fasting glucose, and the use of aspirin/statins/beta blockers. All statistical analyses were performed using the SPSS program (Version 19). A P value <0.05 was considered statistically significant.

3 Results

Clinical and demographic characteristics of the study participants are shown in Table 1. Patients with CVD or risk equivalent had a higher mean age and a higher proportion of men, compared to healthy subjects (P<0.001). Adjusted for differences in age and gender, higher proportions of patients with CVD or risk equivalent had hypertension (P<0.001), hyperlipidemia (P<0.001) and current/past history of smoking (P=0.03). They also had higher mean BMI, waist/hip ratio, mean systolic and diastolic blood pressure, fasting glucose (all P<0.001) and serum triglyceride level (P=0.009), and lower HDL-C level (P<0.001). Mean serum LDL-C level was lower compared to the controls (P=0.001) as explained by the markedly higher proportion of statin (P<0.001) and aspirin users (P<0.001). Mean FMD was markedly lower in patients with CVD or risk equivalent (4.0±2.7 % versus 6.1±4.2 %, P<0.001), with reduced mean circulating EPC (0.71±0.41 versus 0.80±0.37, (log, $\times 10^{-3}$/ml), P=0.004), compared to healthy subjects.

3.1 PR interval prolongation and vascular endothelial dysfunction

PR prolongation >200 ms was more common in patients with CVD or risk equivalent, compared to healthy subjects (45/348 (13 %) versus 4/150 (3 %), P<0.001). Among healthy subjects, PR interval was inversely associated with FMD (Pearson R=−0.20, P=0.015). Among patients with CVD or risk equivalent, PR interval was also inversely associated with FMD (Pearson R=−0.14, P=0.01, Fig. 1). Adjusted for potential confounders (fully adjusted model: age, sex, smoking status, physical activity, body mass index, diabetes mellitus, systolic and diastolic blood pressure, resting pulse rate, LDL-C, HDL-C, triglycerides, fasting

Table 1 Clinical characteristics of the study participants

	CVD patients or risk equivalent (n=348)	Healthy subjects (n=150)	Age–sex adjusted[a] P value
Male (n (%))	232 (67 %)	63 (42 %)	<0.001
Age (years)	65.1±10.2	56.4±10.1	<0.001
Body mass index (kg/m^2)	25.2±3.5	23.7±3.4	<0.001
Waist/hip ratio	0.93±0.06	0.88±0.08	<0.001
Atherothrombotic stroke (n (%))	56 (17 %)	N/A	–
Coronary artery disease (n (%))	236 (68 %)	N/A	–
Diabetes mellitus (n (%))	191 (55 %)	N/A	–
Hypertension (n (%))	227 (66 %)	16 (11 %)	<0.001
Hyperlipidemia (n (%))	210 (62 %)	36 (25 %)	<0.001
Current/Past smoker (n (%))	152 (44 %)	18 (12 %)	0.03
Mean systolic blood pressure (mmHg)	141±18	118±21	<0.001
Mean diastolic blood pressure (mmHg)	80±9	74±9	<0.001
Mean serum LDL level (mmol/l)	2.6±0.7	2.9±0.7	0.001
Mean serum HDL level (mmol/l)	1.3±0.3	1.5±0.4	<0.001
Mean serum triglycerides level (mmol/l)	1.5±1.0	1.2±0.6	0.009
Mean serum fasting glucose (mmol/l)	6.4±2.1	4.9±0.5	<0.001
Mean serum creatinine (mmol/l)	86.9±28.9	73.4±15.1	<0.001
Mean hs-CRP (mg/l)	2.3±4.5	2.2±5.2	0.75
Medications			
Aspirin (n (%))	211 (63 %)	4 (3 %)	<0.001
Statin (n (%))	196 (58 %)	2 (1 %)	<0.001
Beta blockers (n (%))	171 (49 %)	8 (5 %)	<0.001
ACEI/ARB (n (%))	187 (54 %)	1 (1 %)	<0.001
CCB (n (%))	89 (26 %)	7 (5 %)	<0.001
Nitrates (n (%))	89 (26 %)	0 (0 %)	<0.001
Mean FMD (%)	4.0±2.7	6.1±4.2	<0.001
Mean circulating CD133$^+$/KDR$^+$ EPC (log, (×10^{-3}/ml))	0.71±0.41	0.80±0.37	0.004

All values are presented as mean ± SD, except where indicated otherwise

CVD cardiovascular disease, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *FMD* flow-mediated dilatation, *EPC* endothelial progenitor cells

[a]Adjusted for age and sex between patients and healthy subject groups. For age and sex, between-group differences were mutually adjusted for each other

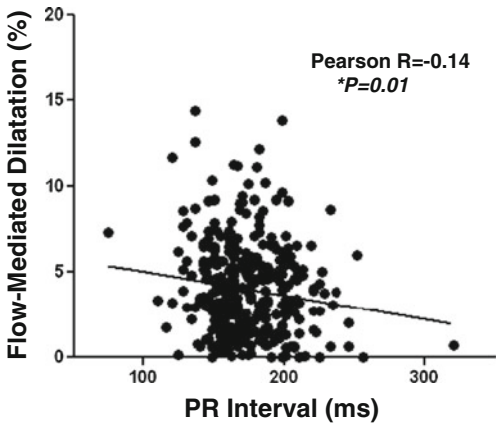

Fig. 1 Relation between flow-mediated dilatation and PR interval in high-risk cardiovascular patients. FMD (%) is inversely associated with PR interval (ms) (Pearson R=−0.14, P=0.01) in 348 high-risk cardiovascular patients

glucose, and the use of aspirin or statins), an increase in PR interval by 80 ms (from the normal cutoff of 120 ms to prolonged cutoff >200 ms) was independently associated with reduced FMD by 1.1 absolute unit (1 %) (B=−0.014, absolute %, (95 % CI −0.027 to −0.002, P=0.026)). Similar results were obtained by repeating the analysis using the crude, adjusted, or fully adjusted models (Table 2).

3.2 PR interval prolongation and circulating CD133$^+$/KDR$^+$ EPC

In patients with CVD or risk equivalent, PR interval was positively associated with circulating CD133$^+$/KDR$^+$ EPCs (Pearson R=+0.14, P=0.009). Circulating CD133$^+$/KDR$^+$ EPC level was significantly increased in patients with PR interval prolongation >200 ms (0.87±0.37 versus 0.68±0.42 (log, ×10^{-3}/ml), P=0.005, Fig. 2). Adjusted for potential confounders (fully adjusted model: age, sex, smoking status, physical activity, body mass index, diabetes mellitus,

Table 2 Crude and adjusted association of PR interval length and parameters of vascular function in patients with cardiovascular disease or risk equivalent (n=348)

	FMD		$CD133^+KDR^+EPC$	
	B (95 % CI)[a]	*P* value	*B* (95 % CI)[a]	*P* value
PR interval (continuous)				
Crude model[b]	−0.014 (−0.025 to −0.003)	0.010	+0.002 (0.001 to 0.004)	0.009
Adjusted model[c]	−0.011 (−0.022 to 0.000)	0.055	+0.002 (0.000 to 0.003)	0.046
Fully adjusted model[d]	−0.014 (−0.027 to −0.002)	0.026	+0.002 (0.000 to 0.004)	0.011
PR prolongation >200 ms (dichotomous)[e]	−0.761 (−1.735 to 0.213)	0.125	+0.186 (0.058 to 0.314)	0.005

Absolute change estimates and 95 % confidence interval of FMD (%) and circulating $CD133^+KDR^+$ EPC (log, ($\times10^{-3}$/ml)) per 1 standard deviation increase in PR interval length were calculated by univariable and multivariable linear regression

FMD flow-mediated dilatation, *EPC* endothelial progenitor cells

[a] 95 % confidence interval in parentheses

[b] PR interval as the only explanatory variable

[c] Adjusted model: FMD, adjusted for potential confounders (age, sex, mean systolic blood pressure, smoking history, and use of statins, beta blockers, calcium channel blockers, or nitrates/nitroglycerine) as defined from unvariable analysis with P value<0.20. EPC, adjusted for age, sex, smoking history, diabetes mellitus, LDL cholesterol, HDL cholesterol, fasting glucose, and use of aspirin, statins, or beta blockers as defined from univariate analysis with P value<0.20

[d] Fully adjusted model: Both FMD and EPC were adjusted for age, sex, smoking status, physical activity, body mass index, diabetes mellitus, systolic and diastolic blood pressure, resting pulse rate, LDL cholesterol, HDL cholesterol, triglycerides, fasting glucose, creatinine, high-sensitivity C-reactive protein, and the use of aspirin, statins, beta blockers, calcium channel blocker, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, or nitrates/nitroglycerine

[e] PR interval prolongation analyzed as a dichotomous explanatory variable with cutoff >200 ms using the fully adjusted model

systolic and diastolic blood pressure, resting pulse rate, LDL-C, HDL-C, triglycerides, fasting glucose, and the use of aspirin or statins), PR prolongation >200 ms remained independently associated with increased $CD133^+/KDR^+$ EPCs by +0.19 (95 % CI 0.06 to 0.31 (log, $\times10^{-3}$/ml), P=0.005). Similar results were consistently obtained by repeating the analysis using the crude, adjusted, or fully adjusted models Table 2). However, PR interval was not associated with circulating $CD133^+/KDR^+$ EPCs among healthy subjects (R=0.05, P=0.58).

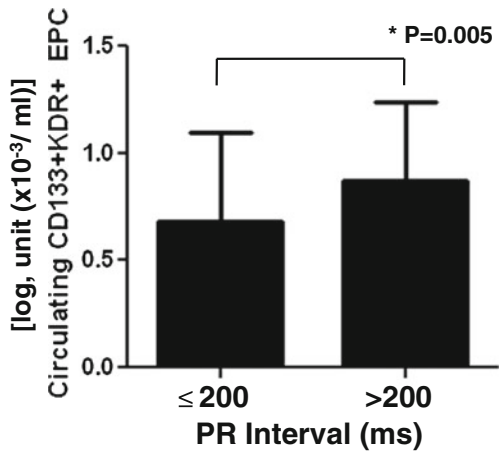

Fig. 2 Relation between circulating $CD133^+/KDR^+$ endothelial progenitor cells and PR interval prolongation in high-risk cardiovascular patients. Circulating $CD133^+/KDR^+$ EPC (log, $\times10^{-3}$/ml) was significantly increased in patients with PR interval prolongation >200 ms (0.87±0.37 versus 0.68±0.42 (log, $\times10^{-3}$/ml), P=0.005) in 348 high-risk cardiovascular patients

4 Discussion

Endothelial dysfunction is a strong and independent predictor of incident cardiovascular events in healthy subjects [13], as well as recurrent cardiovascular events in persons with prior CVD [8]. PR interval prolongation increases CVD risk, although the mechanism is unclear. In this study, we found that among high-risk patients with CVD or risk equivalent, endothelial dysfunction occurred more frequently in patients with PR interval prolongation than those without. Furthermore, after controlling for confounding variables, PR interval remains predictive of endothelial dysfunction. For the first time, we showed that there is heightened vascular repair in patients with CVD with PR interval prolongation versus those without, suggesting that PR interval prolongation further identifies a group with high risk for cardiovascular events over and above traditional risk factors for CVD. Thus, we identified that PR interval prolongation is a risk marker for the continuum of endothelial dysfunction, both in healthy subjects and in those with established CVD, and is a marker for vascular damage and repair. Nevertheless, whether PR interval prolongation is a new independent CVD risk factor will require further studies since it could be an accompanying phenomenon during the

atherosclerosis process together with other residual unadjusted risk factors.

In this current study, we have further extended our prior findings in normal subjects [7] and demonstrated that similar relationship between PR interval prolongation and adverse vascular function changes in patients with prior CVD or risk equivalent. Based on the findings from this and our previous study, the adverse vascular effects associated with PR interval prolongation are similarly observed in healthy subjects as well as in patients with established CVD, and hence, the relations between PR interval prolongation and vascular dysfunction appear consistent in different stages of the cardiovascular continuum. As endothelial dysfunction predicts cardiovascular events, the finding of PR interval prolongation-associated endothelial dysfunction in this study is consistent with the increased risk of atrial fibrillation, pacemaker implantation, and death associated with PR interval prolongation in the community population cohorts of the ARIC and Framingham studies [4, 5]. This is also consistent with the increased risk of heart failure and cardiac death in patients with stable coronary artery disease who had PR interval prolongation in the Heart and Soul Study [6]. The inverse relations between PR interval and vascular endothelial function, as indicated by FMD, is linear based on linear regression analysis and strikingly occurs well below the conventional normal cutoff of PR interval >200 ms. This therefore challenges the long-used conventional cutoff of >200 ms, whether it is justified and reflective of the implicated cardiovascular risk. Notably, the Framingham study also showed that PR interval >149 ms is already associated with increased risk of atrial fibrillation and adverse clinical events [5]. Therefore, the need to further investigate the appropriate definition of a "normal" PR interval is apparent.

The raised circulating $CD133^+/KDR^+$ EPC in patients with PR interval prolongation likely reflects the subclinical vascular injury sustained and the corresponding activation of repair mechanisms as circulating $CD133^+/KDR^+$ EPC has been shown to be raised during acute cardiovascular events and various endogenous and exogenous vascular insults [14]. Although mechanisms underlying the observed findings still remain unclear, PR interval prolongation as a precursor to atrial fibrillation may exert its adverse vascular effects through increased intra-atrial pressure during delayed atrioventricular conduction and subsequent activation of the neurohormonal pathways. Previous studies showed that aldosterone and natriuretic peptides are increased in atrial fibrillation [15] and reversed on cardioversion [16] and maintenance of sinus rhythm [17]. Aldosterone impairs vascular endothelial cell function [18] and plays an important role in hypertension-related vascular dysfunction [19], which is amenable to modulation by renin–angiotensin–aldosterone system blockade [20]. Importantly, recent clinical trial showed that renin–angiotensin–aldosterone blockade prevents atrial fibrillation [21]. If indeed neurohormonal activation is the key mediating mechanism in PR interval prolongation-related vascular dysfunction, findings of our study might have important preventive implications.

4.1 Limitations

This clinical study has been limited by its relatively small sample size and the cross-sectional setting which deters any prospective causal inference and study of mediating mechanisms. Residual confounding is unlikely since conventional cardiovascular risk factors have been adjusted for, and statistical models adopting different criteria had yielded consistently similar results. The coherent findings among healthy and diseased subjects from our current and previous studies [7] suggest that reverse causality is unlikely since such relations were evident prior to occurrence of clinically manifesting events in healthy subjects. Other than circulating EPC, there are other key markers which can strengthen the evidence and mechanism of vascular repair cascade, including *in vivo* re-endothelialization capacity of EPC [22], and plasma markers of angiogenesis such as vascular endothelial growth factor and angiopoietins-1 and -2 [23]. These and markers of neurohormonal activation were not available in the current study and should be further explored. Whether the relations between PR interval and vascular function were truly linear would also need further verification in larger studies. Limitations included the single measurement of PR interval and the overall single estimate of PR interval by computerized analysis from a 12-lead ECG. Also, PR interval and vascular function vary diurnally and are affected by diet, exercise, and medications. Thus, we standardized all measurements to be performed in the morning with overnight fasting including cardiovascular medications to control for these variations. The PR interval estimates as derived from computerized analysis of the overall 12-lead ECG may also not account for possible variations of PR interval across different leads, and whether this may have any clinical implications will require further studies. Based on this study, there is thus a pressing need for further larger-scaled prospective clinical and population-based studies for the demonstration of temporality in terms of vascular function changes with the simultaneous inclusion of hard clinical endpoints.

5 Conclusions

In this study, we conclude that PR interval prolongation in patients with CVD or risk equivalent is associated with worsening endothelial function and raised circulating $CD133^+/KDR^+$ EPC indicating activation of vascular repair mechanisms. These findings lend support to the adverse cardiovascular effects associated with PR interval prolongation and critically challenge the conventional cutoff of normal PR interval defined at 200 ms.

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Conflict of interest None.

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