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CLINICAL RESEARCH

Genetically deprived vitamin D exposure predisposes to atrial fibrillation

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Aims Low vitamin D level is associated with atrial fibrillation (AF) and may be implicated in its pathogenesis.

Methods and results We studied single nucleotide polymorphisms (SNPs) of vitamin D mechanistic pathways and serum 25-hydroxyvitamin D [25(OH)D] levels in an age- and gender-matched case–control study (controls without AF: mean age 68.6 ± 8.7 years, female 25%; $n = 1019$; with AF: mean age 69.7 ± 9.5 years, female 30%; $n = 156$) recruited from a Chinese clinical cohort of patients with stable coronary artery disease. Twelve SNPs involved in the vitamin D mechanistic pathways were studied [biosynthetic: rs4646536, rs10877012, rs3829251, rs1790349; activation: rs2060793, rs1993116; vitamin D-binding protein (VBP)/group-specific component (GC): rs4588, rs7041, rs2282679, rs1155563; and vitamin D receptor: rs1544410, rs10735810]. A genetic risk score (GRS) (0–8) was constructed from SNPs associated with serum 25(OH)D as a proxy to lifelong vitamin D-deficient state. All 4 SNPs involved in the VBP/GC were significantly associated with serum 25(OH)D (rs4588, $P < 0.001$; rs2282679, $P < 0.001$; rs7041, $P = 0.011$; rs1155563, $P < 0.001$; all other SNPs, $P > 0.05$). Vitamin D GRS (points 0–8) generated from these 4 SNPs was independently predictive of serum 25(OH)D [$B = 0.54$, 95% confidence interval (CI) 0.30–0.79; $P < 0.001$]. Genetically deprived vitamin D status as denoted by a low GRS (0–3) independently predicted an increased risk of AF, compared to a high GRS (4–8) (odds ratio = 1.848, 95% CI 1.217–2.805; $P = 0.004$).

Conclusion Genetically deprived vitamin D exposure predisposes to increased AF among patients with coronary artery disease. Whether VBP/GC may alter the risk of AF via alternative mechanisms warrants further studies.

Keywords Vitamin D-binding protein • Genetic polymorphism • 25-Hydroxyvitamin D • Atrial fibrillation

Introduction

Atrial fibrillation (AF), the most common sustained cardiac arrhythmia, remains a leading cause of stroke, death, and health care burden worldwide. Despite identification of important risk factors for AF, a significant fraction of its aetiological risk remains unexplained at large.[1] Vitamin D re-emerged in recent years to have inextricable relations with functional and diseased states of the cardiovascular (CV) system.[2] Deficiency of vitamin D predisposes to systemic inflammation, hypertension, insulin resistance, and activation of prothrombotic cascades, and the renin–angiotensin–aldosterone axis,[3] all of which are mediating mechanisms leading to the final common pathway of CV disease and heart failure.[4]

Recent studies showed that low vitamin D level is associated with AF and may be implicated in its pathogenesis,[5–8] thus suggesting the potential of vitamin D to become a therapeutic target.[9] Nevertheless, vitamin D status is greatly affected by lifestyle, diet, disease status, and seasonal variation,[10] such that a single snapshot of

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What's new?

- Genetic polymorphisms of vitamin D-binding protein are related to altered serum vitamin D status in Chinese patients with coronary artery disease.
- Genetic polymorphisms of vitamin D-binding protein are independently associated with the risk of atrial fibrillation (AF) among Chinese patients with coronary artery disease.
- Inferred genetically deprived vitamin D exposure indicated by a genetic risk score composed from these genetic polymorphisms is associated with increased risk of AF.

serum measurement could hardly help to understand the role of long-term vitamin D exposure on the development of AF. Any causal role of lifelong genetic vitamin D exposure on AF remained unknown. Hence, we investigated whether a novel multi-loci genetic risk score (GRS), a proxy to lifelong-deficient genetic vitamin D exposure, is related to the risk of AF.

Methods

Study population and design

We studied single nucleotide polymorphisms (SNPs) of vitamin D mechanistic pathways and serum 25-hydroxyvitamin D [25(OH)D] level in an age- and gender-matched case–control study (controls without AF: mean age 68.6 ± 8.7 years, female 25%, $n = 1019$; with AF: mean age 69.7 ± 9.5 years, female 30%, $n = 156$) recruited from a Chinese clinical cohort of cardiac outpatients with stable coronary artery disease. Patients with the following conditions were excluded: recent myocardial infarction, unstable angina, coronary revascularization, stroke or acute heart failure within the past 3 months, dilated cardiomyopathy, significant valvular heart disease, liver failure, and clinical/biochemical evidence for concomitant inflammatory disease. The study was structured under the research consortium of the Hong Kong Research Grants Council Theme-Based Research Scheme on Personalized Medicine for Cardiovascular Disease. Further support was obtained from the Health and Medical Research Fund (HMRF) of the Food and Health Bureau, Hong Kong SAR, China. Written informed consent was obtained from all patients. The study was approved by the Institutional Review Board, University of Hong Kong/Hong Kong West Cluster, Hospital Authority.

Baseline demographics and clinical and laboratory assessments

Baseline demographics data, CV risk factors, and medications were documented. Diagnosis of AF was based on 12-lead electrocardiogram and/or 24-h continuous electrocardiogram monitoring, as retrieved and ascertained from the medical records and discharge summaries of the Clinical Management System (CMS), the territory-wide computerized clinical data network of all public hospitals in Hong Kong.

AF was defined as a supraventricular tachyarrhythmia with uncoordinated atrial activity that was characterized by fibrillatory waves variable in magnitude, morphology, and timing and were associated with irregular ventricular response in the background of intact atrioventricular conduction. An AF episode was defined by duration lasting for more than 30 s. Paroxysmal AF refers to AF that spontaneously reverted to sinus rhythm within 7 days from onset. Persistent AF refers to sustained AF beyond 7 days. Permanent AF was defined as uninterrupted AF with duration of >1 year, in which cardioversion failed or was not attempted.[11,12] Coronary artery disease (CAD) was diagnosed in the presence of any of the conditions such as history of myocardial infarction[13], history suggestive of angina pectoris objectively evidenced by inducible ischaemia on exercise treadmill, or single-photon emission computed tomography; the presence of coronary atherosclerosis was defined by coronary angiography, computed tomography (CT), or magnetic resonance imaging. Diagnosis of ischaemic stroke was made on the basis of clinical examinations and CT or magnetic resonance brain imaging.[14] Hypertension was defined as either resting systolic/diastolic blood pressure (BP) ≥140/90 mmHg at two different clinic visits or on medications. Diabetes mellitus was defined as serum fasting glucose ≥7.0 mmol/L or on medications. Hypercholesterolaemia was defined as a fasting total plasma cholesterol level of ≥4.9 mmol/L or on cholesterol-lowering medications. Smoking status was recorded as ever or never smoker. Body mass index (BMI) was calculated as weight in kilograms divided by square of height in metres. Fasting blood was collected for analysis of serum LDL/HDL-cholesterol (LDL/HDL-c), triglycerides, glucose, and creatinine.

Assessment of serum and genetic vitamin D status

Serum 25(OH)D level was measured using validated enzyme immunoassay (Abott/IDS). Vitamin D deficiency was defined as serum 25(OH)D <20 ng/mL. Genotyping was performed using high-throughput Exome chip genotyping technique.

A total of 12 SNPs involved in the vitamin D mechanistic pathways from prior Genome-Wide Association Studies (GWAS) were studied [biosynthetic: rs4646536, rs10877012, rs3829251, rs1790349; activation: rs2060793, rs1993116; vitamin D-binding protein (VBP)/group-specific component (GC): rs4588, rs7041, rs2282679, rs1155563; and vitamin D receptor: rs1544410, rs10735810].[15–24] A composite GRS (linear continuous: 0–8) was constructed based on the summation method as described previously.[25] All risk-conferring alleles across the 4 SNP loci found associated with serum 25(OH)D were assigned a constituent score of 0, 1, or 2 based on allele frequency distribution. The GRS so derived represented a proxy to lifelong vitamin D-deficient state.

Statistical analysis

Continuous variables were presented as mean ± standard deviation. Associations between dichotomous variables were analysed using the χ^2 test. Associations between serum vitamin D, genetic polymorphisms, and other clinical parameters with regard to AF status were examined by univariable and multivariable logistic regression. A multivariable logistic regression model was used to derive odds ratio (OR) for the risk of AF such that each potential confounder variable with a P-value ≤0.10 (based on univariable analysis) was entered. A P-value <0.05 was considered statistically significant. No correction for multiple testing was deemed necessary, as the SNPs under investigation were non-exploratory but included based on prior positive studies. SPSS Statistics (Version 20) was used for the above analyses.

Results

Table 1 presents that patients with AF had matched age and gender distribution with the control subjects. Patients with AF had higher BMI ($P = 0.018$) and were likely to have diabetes ($P = 0.042$) and worse renal function ($P = 0.006$). They also had lower mean diastolic BP and LDL-c (all $P < 0.05$). Patients with AF had lower prevalence of using beta-blockers, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, and lipid-lowering drugs (all $P < 0.001$).

Table 1 Clinical characteristics of CAD patients with AF vs without AF (controls)

	Controls ($n = 1019$)	AF ($n = 156$)	*P*-value
Male, *n* (%)	757 (74)	108 (69)	0.18
Age (years), mean ± SD	68.7 ± 8.6	69.8 ± 9.4	0.12
Ever smoking, *n* (%)	491 (48.2)	68 (43.6)	0.29
Body mass index (kg/m^2), mean ± SD	24.9 ± 3.5	25.9 ± 4.1	0.018*
Diabetes mellitus, *n* (%)	359 (35.2)	68 (43.6)	0.042*
Hypertension, *n* (%)	702 (69.0)	108 (69.2)	0.95
Hyperlipidaemia, *n* (%)	399 (39.2)	48 (30.8)	0.13
Systolic blood pressure (mmHg), mean ± SD	128.5 ± 24.3	124.9 ± 30.1	0.10
Diastolic blood pressure (mmHg), mean ± SD	71.2 ± 14.4	68.2 ± 17.8	0.041*
LDL-cholesterol (mmol/L), mean ± SD	2.41 ± 1.75	2.09 ± 1.79	0.033*
HDL-cholesterol (mmol/L), mean ± SD	0.84 ± 0.81	0.74 ± 0.64	0.16
Triglycerides (mmol/L), mean ± SD	1.21 ± 1.15	1.04 ± 1.15	0.087
Fasting glucose (mmol/L), mean ± SD	6.19 ± 1.92	6.19 ± 1.89	0.98
Creatinine (μmol/L), mean ± SD	106.4 ± 93.6	142 ± 159.4	0.006*
Medications, *n* (%)			
ACEI/ARB	519 (50.9)	48 (30.8)	<0.001*
Beta-blocker	591 (58.0)	58 (37.2)	<0.001*
Calcium channel blocker	323 (31.7)	42 (26.9)	0.230
Lipid lowering	980 (96.2)	133 (85.3)	<0.001*
Season of recruitment, *n* (%)			
Spring	141 (13.8)	16 (10.3)	<0.001*
Summer	158 (15.5)	50 (32.1)	
Autumn	198 (19.4)	21 (13.5)	
Winter	522 (51.2)	69 (44.2)	
Serum 25-hydroxyvitamin D (ng/mL), mean ± SD	23.9 ± 7.6	23.5 ± 8.3	0.54

ACEI, angiotensin-converting enzyme inhibitors; AF, atrial fibrillation; ARB, angiotensin receptor blockers; CAD, coronary artery disease; SD, standard deviation.
*$P < 0.05$.

Serum 25(OH)D at recruitment was not significantly different between AF vs. control subjects ($P = 0.54$).

Genetic polymorphisms of vitamin D mechanistic pathways and serum 25-hydroxyvitamin D level

All 4 SNPs involved in the VBP/GC were significantly associated with serum 25(OH)D in control subjects without AF (rs4588, $P < 0.001$; rs2282679, $P < 0.001$; rs7041, $P = 0.037$; rs1155563, $P < 0.001$). Conversely, other SNPs were not associated with serum 25(OH)D in this control study population ($P > 0.05$).

Vitamin D GRS (points 0–8) generated from the 4 SNPs involved in the VBP/GC was strongly predictive of serum 25(OH)D [$B = 0.52$, 95% confidence interval (CI) 0.294–0.742; $P < 0.001$], and this relation persisted even after multivariable adjustment for potential confounders, including age, gender, BMI, smoking, hypertension, diabetes mellitus, systolic/diastolic BP, triglycerides, LDL/HDL-c, creatinine, use of lipid-lowering drugs, and seasonal variation of recruitment ($B = 0.46$, 95% CI 0.196–0.724; $P = 0.001$).

Genetic polymorphisms of vitamin D-binding protein/group-specific component and risk of AF

Table 2 presents that genetic polymorphisms of VBP/GC found closely linked to serum 25(OH)D levels (rs4588, rs2282679, and rs1155563) were associated with AF (all $P < 0.05$), except rs7041 ($P = 0.48$). Multivariate logistic regression showed that rs4588, rs2282679, and rs1155563, each remained independently associated with AF risk (all $P < 0.05$, *Table 3*).

Aggregated genetic vitamin D exposure, as reflected by the vitamin D GRS composed from SNPs of VBP/GC (rs4588, rs2282679, rs1155563, and rs7041), was significantly associated with reduced risk of AF (OR = 0.91, 95% CI 0.84–0.98; $P = 0.017$). This relation persisted even after multivariable adjustment for potential confounders (OR = 0.88, 95% CI 0.80–0.97; $P = 0.008$, *Table 3*). Categorically, genetically deprived vitamin D status as denoted by a low GRS (0–3) was independently predictive of increased risk for AF, compared to subjects with a high GRS (4–8) (OR = 1.848, 95% CI 1.217–2.805; $P = 0.004$).

Table 2 Genetic polymorphisms of vitamin D mechanistic pathways in CAD patients with AF vs without AF (controls)

	Controls ($n = 1019$)	AF ($n = 156$)	*P*-value
Vitamin D biosynthesis			
rs4646536, *n* (%)			
CC	395 (38.8)	58 (37.2)	0.86
TC	453 (44.5)	73 (46.8)	
TT	171 (16.8)	25 (16.0)	
rs10877012, *n* (%)			
GG	175 (17.2)	25 (16.0)	0.73
GT	455 (44.7)	75 (48.1)	
TT	389 (38.2)	56 (35.9)	
rs3829251, *n* (%)			
GG	510 (50.0)	69 (44.2)	0.20
AG	413 (40.5)	75 (48.1)	
AA	96 (9.4)	12 (7.7)	
rs1790349, *n* (%)			
AA	525 (51.5)	69 (44.2)	0.15
AG	418 (41.0)	77 (49.4)	
GG	76 (7.5)	10 (6.4)	
Vitamin D activation			
rs2060793, *n* (%)			
GG	484 (47.5)	70 (44.9)	0.50
AG	437 (42.9)	74 (47.4)	
AA	98 (9.6)	12 (7.7)	
rs1993116, *n* (%)			
CC	483 (47.4)	71 (45.5)	0.54
TC	436 (42.8)	73 (46.8)	
TT	100 (9.8)	12 (7.7)	
Vitamin D receptor			
rs1544410, *n* (%)			
GG	917 (90.0)	153 (86.5)	0.37
AG	101 (9.9)	21 (13.5)	
AA	1 (0.1)	0 (0)	
rs10735810 (merged into rs2228570), *n* (%)			
GG	293 (28.8)	35 (22.4)	0.23
AG	507 (49.8)	82 (52.6)	
AA	219 (21.5)	39 (25.0)	
VBP/GC			
rs2282679, *n* (%)			
CC	74 (7.3)	11 (7.1)	0.02*
CA	373 (36.6)	75 (48.1)	
AA	572 (56.1)	70 (44.9)	
rs4588, *n* (%)			
AA	72 (7.1)	11 (7.1)	0.02*
AC	374 (36.7)	75 (48.1)	
CC	573 (56.2)	70 (44.9)	
rs1155563, *n* (%)			
CC	114 (11.2)	24 (15.4)	0.03*
TC	443 (43.5)	78 (50)	
TT	462 (45.3)	54 (34.6)	
rs7041, *n* (%)			
TT	496 (48.7)	84 (53.8)	0.48
GT	431 (42.3)	59 (37.8)	
GG	92 (9.0)	13 (8.3)	
Combined genetic risk score, mean ± SD	4.92 ± 2.08	4.49 ± 2.09	0.02*

AF, atrial fibrillation; CAD, coronary artery disease; GC, group-specific component; SD, standard deviation; VBP, vitamin D-binding protein.
*$P < 0.05$.

Table 3 Prediction of genetic polymorphisms of VBP/GC for AF[a]

	Crude model[b]		Multivariable model[c]	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Male, *n* (%)	0.779 (0.539–1.125)	0.18	–	
Age (years)	1.016 (0.996–1.036)	0.119	–	
Body mass index (kg/m^2)	1.073 (1.109–1.131)	*0.008**	1.122 (1.062–1.186)	<0.001*
Ever smoking, *n* (%)	0.831 (0.592–1.167)	0.285	–	
Diabetes mellitus, *n* (%)	1.421 (1.010–1.999)	*0.044**	1.094 (0.707–1.693)	0.69
Hypertension, *n* (%)	1.011 (0.702–1.457)	0.952		
Systolic BP (mmHg)	0.995 (0.989–1.001)	0.098	1.005 (0.992–1.018)	0.45
Diastolic BP (mmHg)	0.988 (0.978–0.998)	*0.017**	0.983 (0.960–1.005)	0.13
LDL-cholesterol (mmol/L)	0.901 (0.819–0.992)	*0.033**	0.939 (0.819–1.078)	0.37
HDL-cholesterol (mmol/L)	0.826 (0.637–1.070)	0.147	–	
Triglycerides (mmol/L)	0.868 (0.738–1.021)	*0.087**	0.975 (0.784–1.213)	0.82
Fasting glucose (mmol/L)	0.999 (0.914–1.091)	0.976	–	
Serum creatinine (μmol/L)	1.002 (1.001–1.002)	*<0.001**	1.001 (0.999–1.002)	0.27
Medications, *n* (%)				
ACEI/ARB	0.428 (0.298–0.615)	*<0.001**	0.476 (0.292–0.777)	0.003*
Beta-blockers	0.429 (0.303–0.607)	*<0.001**	0.503 (0.317–0.798)	0.004*
Calcium channel blockers	0.794 (0.544–1.158)	0.231	–	
Lipid lowering	0.230 (0.133–0.397)	*<0.001**	0.480 (0.227–1.015)	0.055
Serum 25(OH)D (ng/mL)	0.993 (0.971–1.016)	0.542	–	
VBP				
rs2282679		*0.021**		0.002*
CC	Ref.		Ref.	
CA	1.353 (0.685–2.671)		1.802 (0.787–4.125)	
AA	0.823 (0.417–1.625)		0.832 (0.361–1.917)	
rs4588		*0.022**		0.002*
AA	Ref		Ref	
AC	1.313 (0.664–2.594)		1.741 (0.758–3.998)	
CC	0.800 (0.405–1.580)		0.806 (0.349–1.863)	
rs1155563		*0.034**		0.018*
CC	Ref		Ref	
TC	0.836 (0.506–1.381)		0.809 (0.439–1.492)	
TT	0.555 (0.329–0.936)		0.456 (0.238–0.871)	
rs7041		0.484		0.29
TT	Ref		Ref	
GT	0.808 (0.566–1.155)		0.709 (0.455–1.104)	
GG	0.834 (0.447–1.559)		0.759 (0.351–1.643)	
Combined genetic score (0–8)[d]	0.909 (0.840–0.983)	*0.017**	0.880 (0.801–0.966)	0.008*
Combined genetic score<4 (vs. 4–8)	1.545 (1.102–2.167)	*0.012**	1.848 (1.217–2.805)	0.004*

ACEI, angiotensin-converting enzyme inhibitors; AF, atrial fibrillation; ARB, angiotensin receptor blockers; CI, confidence interval; GC, group-specific component; OR, odd ratio; VBP, vitamin D-binding protein.

[a]OR estimates and 95% confidence intervals of AF explained by variable of interest as estimated by univariable and multivariable logistic regression.

[b]Unadjusted estimates.

[c]Adjusted for potential confounders as defined from univariable analysis with *P*-value ≤0.10.

[d]Combined linear genetic score (0–8) based on combined allele score summation from 4 constituent single nucleotide polymorphisms of VBP/GC (rs2282679, rs4588, rs1155563, and rs7041).

*$P < 0.05$.

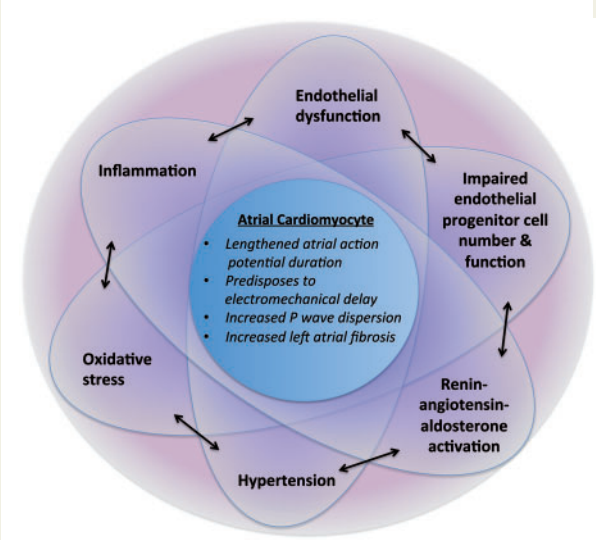

Figure 1 Schematic diagram of the pathophysiological mechanisms of vitamin D deficiency leading to atrial fibrillation. The depicted mechanisms were either through direct effects on the atrial cardiomyocyte or by altering systemic/micro-environmental factors including hypertension, oxidative stress, inflammation, endothelial dysfunction and repair, and neurohormonal activation.

Discussion

Clinical and epidemiological studies showed that vitamin D has inextricable relations with the CV system in health and disease.[2] Nevertheless, controversies continued regarding the exact role of vitamin D in this regard, whether represented a bystander-only indicator or a causal culprit that is amenable to treatment. More recently, the potential role of vitamin D in arrhythmogenesis, particularly AF, was recognized.[26]

To the best of our knowledge, this is the first clinical study that investigated the role of genetic vitamin D exposure on the risk of AF. In our study, genetic exposure to vitamin D was independently associated with reduced risk of AF. Categorically, genetically deprived vitamin D status as denoted by a low GRS (0–3) had an excess AF risk by 85% compared to those with a high GRS (4–8). This suggested that vitamin D deficiency might have an important role in AF development, in coherence with earlier clinical studies[5–7,27] and a meta-analysis[8] relating low serum vitamin D to AF.

The mechanisms through which vitamin D alters the risk of AF must be via either its direct actions on the atrium or indirect modulation of CV risk factors and micro-environmental factors (*Figure* 1). Animal studies showed that 1, 25(OH)D has direct electromechanical effects on the atrium, resulting in dose-dependent lengthening of the atrial action potential duration, providing arrhythmic substrate for AF. It was shown that 1, 25(OH)D could experimentally prevent or terminate AF.[28] Clinically, vitamin D deficiency was associated with electrocardiographic P-wave dispersion, as well as atrial electromechanical delay measured by tissue Doppler imaging, which ameliorates on vitamin D replacement.[29] Furthermore, low serum 25(OH)D levels have been found to be associated with more extensive left atrial fibrosis among patients with lone paroxysmal AF who underwent cryoballoon catheter ablation, which may alter the risk of AF recurrence post-ablation.[30]

Alternatively, the links of vitamin D deficiency to endothelial dysfunction and progenitor cell counts/activity,[31,32] systemic inflammation, raised oxidative stress, systemic hypertension, insulin resistance, and renin–angiotensin–aldosterone activation,[3] as well as their sequelae of heart failure and CAD, are all important causes of AF.

It was worth noting that, not unexpectedly, there was no association between serum 25(OH)D and AF from this case–control study. Vitamin D status is closely linked to dietary and lifestyle patterns that frequently changed after clinically manifest cardiac disease, and its *post hoc* measured level may no longer reflect the preceding exposure, rendering any genuine case–control differences undetectable. The study of genetic variants on top of serum biomarkers helped to minimize any effect of reverse causality. In our study, the presence of AF was associated with higher BMI, worse renal function, lower diastolic BP and LDL-c, and lower prevalence of using beta-blockers, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, and lipid-lowering drugs. Some of these factors could be associated with vitamin D status. We tried to eliminate any confounding effects by adjusting these potential confounders in the multivariable regression model, and the study findings remained unchanged. Of note, although beta-blockers were expected to be commonly used among patients with AF for rate control, the study sample comprised patients with stable CAD among whom the use of beta-blockers was already common for the purpose for anti-anginal or anti-hypertensive effects. Furthermore, coexistence of bradycardia/sick sinus syndrome among AF patients may preclude their use of beta-blockers. Data regarding this are lacking in this study and is one of the limitations.

Limitations

There are several other limitations of this study. First, this is a case–control study inherently prone to recall and selection bias. Despite our meticulous strategies to reduce reverse causality and confounding, further large prospective studies will be needed to confirm such findings. Secondly, the rather selected high-risk group of our subjects with CAD may limit the generalizability of the study findings and potentially may not apply to low-risk populations. Thirdly, ethnic difference in genetics should also be taken into consideration. Finally, mechanistic studies are lacking, and this should be further addressed in the future.

As the most common clinically encountered sustained cardiac arrhythmia, AF represents severe burdens of death and health care burden worldwide. The idea to alter such health care burden via treating a highly prevalent and reversible nutritional deficiency as in the case of vitamin D remained highly attractive. A well-designed and adequately powered randomized controlled trial to study the clinical preventive effects of vitamin D for AF and related hard endpoints is warranted.

Conclusions

Genetically deprived vitamin D exposure predisposes to increased risk of AF in CAD patients. Whether VBP/GC may alter AF risk via alternative mechanisms warrants further studies.

Conflict of interest: none declared.

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References

1. Lip GY, Tse HF, Lane DA. Atrial fibrillation. *Lancet* 2012;**379**:648–61.
2. Norris KC, Williams SF. Race/ethnicity, serum 25-hydroxyvitamin D, and heart disease. *JAMA* 2013;**310**:153–5.
3. Norman PE, Powell JT. Vitamin D and cardiovascular disease. *Circ Res* 2014;**114**: 379–93.
4. Pilz S, Marz W, Wellnitz B, Seelhorst U, Fahrleitner-Pammer A, Dimai HP. Association of vitamin D deficiency with heart failure and sudden cardiac death in a large cross-sectional study of patients referred for coronary angiography. *J Clin Endocrinol Metab* 2008;**93**:3927–35.
5. Chen WR, Liu ZY, Shi Y, Yin DW, Wang H, Sha Y *et al*. Relation of low vitamin D to nonvalvular persistent atrial fibrillation in Chinese patients. *Ann Noninvasive Electrocardiol* 2014;**19**:166–73.
6. Ozcan OU, Gurlek A, Gursoy E, Gerede DM, Erol C. Relation of vitamin D deficiency and new-onset atrial fibrillation among hypertensive patients. *J Am Soc Hypertens* 2015;**9**:307–12.
7. Emren SV, Aldemir M, Ada F. Does deficiency of vitamin D increase new onset atrial fibrillation after coronary artery bypass grafting surgery? *Heart Surg Forum* 2016;**19**:E180–4.
8. Zhang Z, Yang Y, Ng CY, Wang D, Wang J, Li G *et al*. Meta-analysis of vitamin D deficiency and risk of atrial fibrillation. *Clin Cardiol* 2016;**39**:537–43.
9. Chen M, Yu L, Liu Q, Jiang H, Zhou S. Vitamin D: a potential important therapeutic target for atrial fibrillation. *Int J Cardiol* 2015;**198**:91–2.
10. Xu C, Perera RA, Chan YH, Fang VJ, Ng S, Ip DK *et al*. Determinants of serum 25-hydroxyvitamin D in Hong Kong. *Br J Nutr* 2015;**114**:144–51.
11. Fuster V, Rydén LE, Cannom DS, Crijns HJ, Curtis AB, Ellenbogen KA *et al*. ACC/AHA/ESC 2006 guidelines for the management of patients with atrial fibrillation: full text. *Europace* 2006;**8**:651–745.
12. Fuster V, Ryden LE, Cannom DS, Crijns HJ, Curtis AB, Ellenbogen KA *et al*. 2011 ACCF/AHA/HRS focused updates incorporated into the ACC/AHA/ESC 2006 Guidelines for the management of patients with atrial fibrillation: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines developed in partnership with the European Society of Cardiology and in collaboration with the European Heart Rhythm Association and the Heart Rhythm Society. *J Am Coll Cardiol* 2011;**57**:e101–98.
13. Antman E, Bassand J-P, Klein W, Ohman M, Lopez Sendon JL, Rydén L *et al*. Myocardial infarction redefined—a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. *J Am Coll Cardiol* 2000;**36**:959–69.
14. Adams H, Adams R, Del Zoppo G, Goldstein LB. Guidelines for the early management of patients with ischemic stroke: 2005 guidelines update a scientific statement from the Stroke Council of the American Heart Association/American Stroke Association. *Stroke* 2005;**36**:916–23.
15. Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D *et al*. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. *Lancet* 2010;**376**:180–8.
16. Elkum N, Alkayal F, Noronha F, Ali MM, Melhem M, Al-Arouj M *et al*. Vitamin D insufficiency in Arabs and South Asians positively associates with polymorphisms in GC and CYP2R1 genes. *PLoS One* 2014;**9**:e113102.
17. Yokoyama K, Nakashima A, Urashima M, Suga H, Mimura T, Kimura Y *et al*. Interactions between serum vitamin D levels and vitamin D receptor gene FokI polymorphisms for renal function in patients with type 2 diabetes. *PLoS One* 2012;**7**:e51171.
18. Engelman CD, Fingerlin TE, Langefeld CD, Hicks PJ, Rich SS, Wagenknecht LE *et al*. Genetic and environmental determinants of 25-hydroxyvitamin D and 1, 25-dihydroxyvitamin D levels in Hispanic and African Americans. *J Clin Endocrinol Metab* 2008;**93**:3381–8.
19. Batai K, Murphy AB, Shah E, Ruden M, Newsome J, Agate S *et al*. Common vitamin D pathway gene variants reveal contrasting effects on serum vitamin D levels in African Americans and European Americans. *Hum Genet* 2014;**133**: 1395–405.
20. Lu L, Sheng H, Li H, Gan W, Liu C, Zhu J *et al*. Associations between common variants in GC and DHCR7/NADSYN1 and vitamin D concentration in Chinese Hans. *Hum Genet* 2012;**131**:505–12.
21. Bailey R, Cooper JD, Zeitels L, Smyth DJ, Yang JH, Walker NM *et al*. Association of the vitamin D metabolism gene CYP27B1 with type 1 diabetes. *Diabetes* 2007; **56**:2616–21.
22. Hama T, Norizoe C, Suga H, Mimura T, Kato T, Moriyama H *et al*. Prognostic significance of vitamin D receptor polymorphisms in head and neck squamous cell carcinoma. *PLoS One* 2011;**6**:e29634.
23. Signorello LB, Shi J, Cai Q, Zheng W, Williams SM, Long J *et al*. Common variation in vitamin D pathway genes predicts circulating 25-hydroxyvitamin D Levels among African Americans. *PLoS One* 2011;**6**:e28623.
24. Singh A, Falk MK, Subhi Y, Sørensen TL, Mori K. The association between plasma 25-hydroxyvitamin D and subgroups in age-related macular degeneration: a cross-sectional study. *PLoS One* 2013;**8**:e70948.
25. Smith JA, Ware EB, Middha P, Beacher L, Kardia SL. Current applications of genetic risk scores to cardiovascular outcomes and subclinical phenotypes. *Curr Epidemiol Rep* 2015;**2**:180–90.
26. Thompson J, Nitiahpapand R, Bhatti P, Kourliouros A. Vitamin D deficiency and atrial fibrillation. *Int J Cardiol* 2015;**184**:159–62.
27. Belen E, Aykan A, Kalay E, Sungur M, Sungur A, Cetin M. Low-level vitamin D is associated with atrial fibrillation in patients with chronic heart failure. *Adv Clin Exp Med* 2016;**25**:51–7.
28. Hanafy D, Chang S-L, Lu Y-Y, Chen Y-C, Kao Y-H, Huang J-H *et al*. Electromechanical effects of 1, 25-dihydroxyvitamin d with antiatrial fibrillation activities. *J Cardiovasc Electrophysiol* 2014;**25**:317–23.
29. Canpolat U, Yayla Ç, Akboğa M, Özcan E, Turak O, Özcan F *et al*. Effect of vitamin D replacement on atrial electromechanical delay in subjects with vitamin D deficiency. *J Cardiovasc Electrophysiol* 2015;**26**:649–55.
30. Canpolat U, Aytemir K, Hazirolan T, Ozer N, Oto A. Relationship between vitamin D level and left atrial fibrosis in patients with lone paroxysmal atrial fibrillation undergoing cryoballoon-based catheter ablation. *J Cardiol* 2017;**69**: 16–23.
31. Chan YH, Lau KK, Yiu KH, Li SW, Tam S, Lam TH *et al*. Vascular protective effects of statin-related increase in serum 25-hydroxyvitamin D among high-risk cardiac patients. *J Cardiovasc Med (Hagerstown)* 2015;**16**:51–8.
32. Yiu YF, Chan YH, Yiu KH, Siu CW, Li SW, Wong LY *et al*. Vitamin D deficiency is associated with depletion of circulating endothelial progenitor cells and endothelial dysfunction in patients with type 2 diabetes. *J Clin Endocrinol Metab* 2011;**96**:E830–5.