

## RESEARCH ARTICLE

# Vitamin D levels and vitamin D receptor variants are associated with chronic heart failure in Chinese patients

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**Background:** Vitamin D is an indispensable molecule for human health. Wide ranges of diseases are linked with vitamin D deficiencies. Role of vitamin D in chronic heart failure has been demonstrated in different populations; however, reports are limited in Chinese population. Vitamin D exerts its effect through vitamin D receptor and variants in vitamin D receptor (VDR) gene are shown to affect vitamin D signaling. In the present study, we hypothesized that both vitamin D levels and VDR variants could be associated with the development of chronic heart failure.

**Materials and Methods:** We enrolled 145, chronic heart failure patients those admitted to Department of Cardiothoracic Surgery, Beijing Luhe Hospital of Capital Medical University and fulfilled NYHA inclusions criteria. In addition, ninety healthy subjects from similar geographical location were enrolled as healthy controls. Plasma levels of vitamin D were quantified by ELISA. VDR variants (*BsmI*, *Apal*, *TaqI*, and *FokI*) were genotyped by PCR-RFLP.

**Results:** Plasma levels of vitamin D were significantly lower in chronic heart patients compared to healthy controls. Heterozygous and minor allele for *FokI* and *TaqI* polymorphisms were significantly higher in heart failure patients when compared to healthy controls. In addition, combined analysis of vitamin D levels and VDR mutants revealed association of vitamin D deficiencies and VDR mutants with chronic heart failure.

**Conclusions:** The results of the present investigation showed an important role of vitamin D and VDR variants with chronic heart failure.

**KEYWORDS**

Chinese, heart failure, polymorphism, vitamin D receptor, vitamin D

## 1 | INTRODUCTION

About 100 year back, Elmer McCollum administrated cod-liver oil to the rickets dogs and demonstrated improvement in clinical phenotype and named the constituent as vitamin D.<sup>1</sup> Role of vitamin D in human health has been well documented, and deficiency of vitamin D has been linked with wide range of diseases such as rickets, osteomalacia,

bone-related disorders, cancer, autoimmune disorders, different infectious diseases, diabetes, hypertension, and heart disease.<sup>2,3</sup>

Heart failure is one of the serious syndromes affects 1%-2% of adult population in the developed countries.<sup>4</sup> About 6 lakhs American died per year and this rate is one-fourth of total death in the country (<https://www.cdc.gov/heartdisease/facts.htm>). In China, prevalence of HF was 0.9% which is lower than data reported from western world.<sup>5</sup> Older

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age group had higher prevalence of HF compared to younger individuals.<sup>4</sup> Interestingly, Chinese females are more susceptible to HF than male which contrasts with developed countries data. The exact etiology of heart failure is not known; however, various factors have been attributed for the development of HF such as dysfunctional endocrine and metabolic functions, various infections, inflammatory disorders, and some genetic variations.<sup>6</sup> In developed world, myocardial infarction and hypertension remained major contributors of heart failures. However, majority of heart failure patients had hypertension when investigated in North American, Australian, and European population.<sup>4</sup>

Role of vitamin D in heart failure has been elegantly demonstrated in both human and animal model system.<sup>7</sup> A recent double-blind controlled trial showed improvement in ejection fraction of heart failure patients suggesting importance of vitamin D status determining clinical phenotype of heart failure.<sup>8</sup> Furthermore, a recent investigation showed repletion of vitamin D improves quality of life and normalize levels of parathyroid hormone and C-reactive protein.<sup>9</sup> A meta-analysis including previously published papers revealed lower serum parathyroid hormone and inflammatory molecules on vitamin D supplementations compared to patients without vitamin D therapy.<sup>10</sup> These observations combinedly suggest an important role of vitamin D in heart failure pathogenesis.

Vitamin D receptor (VDR) is essential for vitamin D signaling cascade. VDR expression levels on cells or three-dimensional structure of VDR determine effective signaling outcome. Variants in VDR coding region may affect protein structure and ultimately hamper binding of vitamin D that may lead to limited signaling. Mutation in promoter region possibly harms or increased binding of various transcription factors and affect transcriptional levels of messenger RNA of VDR gene. Various common single nucleotide polymorphisms have been reported in the literature; however, four SNPs (*FokI* [rs2228570], *TaqI* [rs731236], *BsmI* [rs1544410], and *Apal* [rs7975232]) have been widely investigated. These variants (*FokI*, *TaqI*, *BsmI*, and *Apal*) are believed to controlled vitamin D signaling; however, reports are contradictories. A very recent study showed association of *FokI* and *TaqI* polymorphism with plasma levels of 25-OH vitamin D<sub>3</sub>.<sup>11</sup>

In the present study, we aimed to investigate role of vitamin D<sub>3</sub> in susceptibility to chronic heart failure. As vitamin D exerts its affect through VDR, we further hypothesized that genetic variation in VDR gene would be associated with predisposition to chronic heart failure. To achieve our objective, we enrolled heart failure patients and healthy controls hailing from China, quantified plasma levels of 25-OH vitamin D<sub>3</sub>, genotyped four common polymorphisms of VDR gene (*FokI*, *TaqI*, *BsmI*, and *Apal*), and correlate findings with susceptibility/resistance to development of heart failure.

## 2 | MATERIALS AND METHODS

### 2.1 | Patients and controls

One hundred and forty-five chronic heart failure patients were enrolled in the present study. All patients those admitted to Department of Cardiothoracic Surgery, Beijing Luhe Hospital of

Capital Medical University, were subjected to electrocardiogram, chest X-ray, echo-Doppler study, clinically accessed by trained clinician, and those fulfilled New York Heart Association criteria's were included in the present investigation. In brief, chronic heart failure cases were diagnosed by intensive history investigation, physical examination by expert physician, scoring of impaired Left Ventricular (LV) systolic function (LV ejection fraction  $\leq$  40%) and LV dilation (LV end-diastolic diameter  $>$  5.5 cm) by echocardiography. Patients belonged to all classes of NYHA (I, II, III, and IV) and essentially  $\geq$ 18 years of age were included in the present study. All patients were treated with standard treatment protocol as suggested by American Heart Association<sup>12</sup> and European society of Heart study.<sup>13</sup> Patients with autoimmune diseases, acute coronary syndromes, liver dysfunctions, chronic infections, and malignancy were excluded from the present study. Age- and sex-matched ninety healthy subjects hailing from similar geographical area were enrolled as controls. All controls are essentially healthy and no history of any heart-related anomalies. Furthermore, healthy controls with history of autoimmune diseases and hypertension were excluded from the study. The study protocol was approved by Institutional Human Ethical Committee of Beijing Luhe Hospital of Capital Medical University, and written informed consent was obtained from all subjects. About 4 mL of intravenous blood samples were collected from each participant with EDTA, mixed thoroughly and centrifuges at 400 g for 20 minutes for isolation of plasma. Isolated plasmas were stored at  $-80^{\circ}\text{C}$  till use for quantification of cytokines. Whole blood was used for isolation genomic DNA by kit (SIGMA-ALDRICH, Hong Kong, China) as per manufacturer's instructions.

### 2.2 | Vitamin D<sub>3</sub> quantification

Plasma samples were thawed, and levels of 25-OH vitamin D<sub>3</sub> was quantified by enzyme-linked immunosorbent assay (ELISA) according to manufacturer's protocol (R&D system, Minneapolis, MN, USA). To test levels of vitamin D in both patients and healthy controls, we quantified plasma levels 25-OH vitamin D for its longer half-life and least affected by exogenous vitamin D.

### 2.3 | VDR polymorphism genotyping

As described earlier,<sup>11,14</sup> vitamin D receptor polymorphisms (*FokI*, *TaqI*, *BsmI*, and *Apal*) were genotyped by polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP). Thermocycling condition for amplification of PCR fragment was as follows: initial denaturation for 5 minutes at  $95^{\circ}\text{C}$ , followed by 35 cycles of denaturation at  $95^{\circ}\text{C}$  for 40 seconds, annealing at  $60^{\circ}\text{C}$  for 45 seconds, and extension at  $72^{\circ}\text{C}$  for 45 seconds. The amplified products were digested by respective enzymes and analyzed by gel electrophoresis. In addition, about 20% of samples were randomly picked up and sequenced by direct sequencing. The results of PCR-RFLP technique and sequencing observations were absolute concordant.

## 2.4 | Statistical analysis

GraphPad prism 6.03 (San Diego, CA, USA) was used for all statistical analysis. Mean plasma vitamin D<sub>3</sub> concentration in healthy control and CHF patients was compared by Student's *t* test. Distribution of VDR polymorphisms among controls and CHF patients was analyzed by Fisher's exact test. A *P* value <0.05 was taken as significant, however genotype comparison a *P* value <0.001 (0.05/4) considered as significant after Bonferroni correction for four SNPs. Bonferroni correction is essential for minimizing type-I errors when several statistical test carried out in single data set.<sup>15</sup> To calculate *P* value after Bonferroni correction, divide critical *P* value ( $\alpha$ : 0.05) by total number SNPs considered in the present study, that is, four.

## 3 | RESULTS

### 3.1 | Baseline characteristics

In the present hospital-based case-controls investigation, 145 chronic heart failure patients were enrolled. Out of them, 102 were male and female were 30%. The mean age of patients was 52.67 with a standard deviation of 15.37 years. In addition, 90 healthy subjects were recruited as controls. The male-female controls were 63 and 27, respectively. The mean age of controls was 53.24 ( $\pm$ 12.83 SD) and comparable with those of patients.

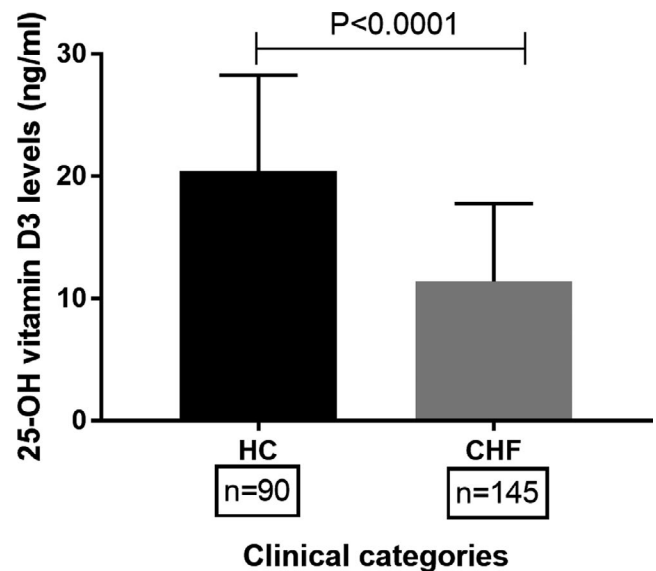
### 3.2 | Plasma 25-OH vitamin D<sub>3</sub> levels in patients and controls

Plasma levels of 25-OH vitamin D<sub>3</sub> in healthy controls and heart failure patients were quantified by ELISA. Mean vitamin D<sub>3</sub> levels in chronic heart failure patients and healthy controls were compared by Student's *t* test. As shown in Figure 1, CHF patients displayed significantly lower levels of plasma vitamin D<sub>3</sub> compared to healthy controls (*P* < 0.0001).

Further, based on 25-OH vitamin D<sub>3</sub> levels all subjects were segregated into three major groups (a) sufficient (subjects with more than 30 ng/mL concentration), (b) insufficient (25-OH vitamin D<sub>3</sub> levels 10-30 ng/mL), and (c) deficient (individuals having <10 ng/mL) and their distribution was investigated among healthy controls and patients. As shown in Table 1, 25-OH vitamin D<sub>3</sub> deficient were more prevalent in chronic heart failure patients (28%) compared to healthy controls (15%). Furthermore, individuals with insufficient vitamin D levels were highly frequent in patients then controls; however, this difference was could not reach significant level (*P* = 0.06).

### 3.3 | Prevalence VDR polymorphism in healthy controls

Vitamin D receptor polymorphisms in healthy controls (*n* = 90) were genotyped by PCR-RFLP, and the observations are depicted in Table 2. In line with earlier reports, we observed higher abundance of wild type in studied healthy subjects (*FokI*: 64%, *TaqI*: 63%, *BsmI*: 83%), followed by heterozygous (*FokI*: 32%, *TaqI*: 35%, *BsmI*: 14%)



**FIGURE 1** Plasma levels of 25-OH vitamin D<sub>3</sub> in healthy controls and chronic heart failure patients. Plasma levels of 25-OH vitamin D<sub>3</sub> were quantified in healthy controls (*n* = 90) and chronic heart failure patients (*n* = 145) by ELISA according to manufacturer's instructions. Mean levels of 25-OH vitamin D<sub>3</sub> in two clinical categories were compared by Student's *t* test. A *P* value <0.05 was considered as significant

and homozygous remained least one (*FokI*: 4%, *TaqI*: 2%, *BsmI*: 3%). However, higher prevalence of heterozygous was more frequent (42%) for *Apal* polymorphism in comparison with wild type (41%) and homozygous mutant (17%). The allele and genotype frequencies remained constant from generation to generation in the absence of any external evolutionary force defined by Hardy-Weinberg equilibrium (HWE).<sup>16</sup> Distribution of genotypes for all VDR polymorphism was in HWE (*FokI*:  $\chi^2 = 0.07$ , *P* = 0.78; *TaqI*:  $\chi^2 = 0.89$ , *P* = 0.34; *BsmI*:  $\chi^2 = 2.17$ , *P* = 0.14 and *Apal*:  $\chi^2 = 0.93$ , *P* = 0.33).

### 3.4 | Distribution of VDR polymorphisms in patients and healthy subjects

Four common polymorphisms of VDR gene in patients and controls were genotyped by PCR-RFLP, and data are shown in Table 2. Prevalence of heterozygous mutant (Ff) and minor allele (f) for *FokI* polymorphism was significantly higher in chronic heart failure patients compared to healthy controls (Ff: *P* < 0.0001, OR = 3.42; f: *P* < 0.0001, OR = 2.45). Similar trend was also noticed for *TaqI* gene polymorphism; Tt genotype and allele "t" were more frequent in patient then controls (Tt: *P* = 0.002, OR = 2.35; t: *P* = 0.007, OR = 1.86). Distributions of other VDR polymorphisms (*BsmI* and *Apal*) in patients and controls were comparable, and no significant difference was noticed.

### 3.5 | Combined distribution of VDR polymorphisms and 25-OH vitamin D<sub>3</sub> levels

Vitamin D receptor is an important component in vitamin D signaling pathways as vitamin D employs VDR for its effect. Functional

25-OH Vitamin D <sub>3</sub> status	Healthy controls (n = 90)	CHF (n = 145)	P value	OR (95% CI)
Sufficient (>30 ng/mL)	11 (12)	6 (4)	Ref	1
Insufficient (10-30 ng/mL)	66 (73)	99 (68)	0.06	2.75 (0.94-7.76)
Deficient (<10 ng/mL)	13 (15)	40 (28)	0.003	5.64 (1.72-17.68)

**TABLE 1** Distribution of 25-OH vitamin D<sub>3</sub> in patients and controls

CHF, chronic heart failure patients; CI, confidence interval; HC, healthy control; NS, Not significant; OR, odds ratio. Data are no. (%) of participants unless otherwise specified.

**TABLE 2** Distribution of VDR polymorphisms in healthy controls and chronic heart failure patients

VDR Polymorphisms	Genotype or Allele	HC (n = 90)	CHF (n = 145)	P value	OR (95% CI)
FokI (rs2228570)	Genotype				
	FF	58 (64)	49 (34)	1	Ref
	Ff	29 (32)	84 (58)	<0.0001	3.42 (1.94-6.07)
	ff	3 (4)	12 (8)	0.02	4.73 (1.35-16.27)
	Allele				
	F	145 (81)	182 (63)	1	Ref
	f	35 (19)	108 (37)	<0.0001	2.45 (1.60-3.77)
TaqI (rs731236)	Genotype				
	TT	57 (63)	61 (42)	1	Ref
	Tt	31 (35)	78 (54)	0.002	2.35 (1.37-4)
	tt	2 (2)	6 (4)	0.28	2.80 (0.66-14.01)
	Allele				
	T	145 (81)	200 (69)	1	Ref
	t	35 (19)	90 (31)	0.007	1.86 (1.19-2.89)
BsmI (rs1544410)	Genotype				
	BB	75 (83)	115 (79)	1	Ref
	Bb	13 (14)	25 (17)	0.58	1.25 (0.60-2.50)
	bb	2 (3)	5 (4)	0.70	1.63 (0.33-8.34)
	Allele				
	B	163 (91)	255 (88)	1	Ref
	b	17 (9)	35 (12)	0.45	1.31 (0.72-2.39)
ApaI (rs7975232)	Genotype				
	AA	37 (41)	55 (38)	1	Ref
	Aa	38 (42)	68 (47)	0.55	1.20 (0.68-2.11)
	aa	15 (17)	22 (15)	1	0.98 (0.45-2.07)
	Allele				
	A	112 (62)	178 (61)	1	Ref
	a	68 (38)	112 (39)	0.92	1.03 (0.71-1.52)

CI, confidence interval; HC, healthy control; NS, Not significant; OR, odds ratio. Data are no. (%) of participants unless otherwise specified.

variants in VDR genes which affect transcription rate of VDR messenger RNA or effect three-dimensional structure of VDR would affect vitamin D signaling process. We hypothesized that combined 25-OH vitamin D<sub>3</sub> levels and VDR polymorphism may be associated with susceptibility to development of CHF. As we observed

genetic association of *FokI* and *TaqI* variants with susceptibility to CHF, in the combined analysis only those two polymorphisms were considered. As shown in Table 3, subjects with Ff/sufficient, Ff/insufficient, ff/insufficient, and Ff/deficient were significantly higher in chronic heart failure patients compared to healthy controls. In

**TABLE 3** Distribution of *FokI* and *TaqI* polymorphisms and vitamin D status in healthy controls and chronic heart failure patients

VDR polymorphism/ vitamin D status	HC (n = 90)	CHF (n = 145)	P value	OR (95% CI)
<i>FokI</i> polymorphism/vitamin D status				
FF/Sufficient	11 (13)	1 (1)		Ref
Ff/Sufficient	0	6 (4)	0.0004	Infinity (5.60 to infinity)
ff/Sufficient	0	0		Not calculated
FF/insufficient	43 (48)	48 (33)	0.004	12.28 (1.96-134.9)
Ff/insufficient	21 (23)	38 (26)	0.0007	19.9 (2.91-219.4)
ff/insufficient	2 (2)	12 (8)	0.0002	66 (4.92-746.2)
FF/deficient	4 (4)	0	1	0 (0-27)
Ff/deficient	8 (9)	40 (28)	<0.0001	55 (6.62-602)
ff/deficient	1 (1)	0	1	0 (0-108)
<i>TaqI</i> polymorphism/vitamin D status				
TT/Sufficient	11 (13)	1 (1)		Ref
Tt/Sufficient	0	5 (3)	0.001	Infinity (4.26 to infinity)
tt/Sufficient	0	0		
TT/insufficient	39 (43)	46 (32)	0.003	12.97 (2.05-142.7)
Tt/insufficient	26 (28)	47 (32)	0.0003	19.88 (3.03-218.3)
tt/insufficient	1 (1)	6 (4)	0.001	66 (3.75-789.6)
TT/deficient	7 (8)	14 (10)	0.002	22 (2.87-250.6)
Tt/deficient	5 (6)	26 (18)	<0.0001	57.2 (7.25-630.6)
tt/deficient	1 (1)	0	1	0 (0-108)

CHF, chronic heart failure patients; CI, confidence interval; HC, healthy control; NS, Not significant; OR, odds ratio. Data are no. (%) of participants unless otherwise specified.

contrast, the prevalence of FF/insufficient combination was higher in controls compared to patients. Similar observation was observed for combination analysis of *TaqI* variants and plasma levels of 25-OH vitamin D<sub>3</sub> levels; Tt/sufficient, Tt/insufficient, tt/insufficient, TT/deficient, and Tt/deficient were significantly higher in CHF patients when compared to healthy controls. However, TT/insufficient combination was more frequent in controls than patients.

## 4 | DISCUSSION

In the present investigation, we observed significantly lower levels of plasma 25-OH vitamin D<sub>3</sub> in chronic heart failure patients compared to healthy controls. Heterozygous and minor allele of VDR (*FokI* and *TaqI*) polymorphisms were more frequent in heart failure patients with references to healthy controls. Interestingly, combined analysis of plasma 25-OH vitamin D<sub>3</sub> and VDR polymorphisms indicated importance of simultaneous investigation of these duos in genetic association studies for determination of susceptibility/resistance factor against disease development.

25-OH vitamin D<sub>3</sub> is the best indicator of vitamin D status in an individual. Various studies have been carried out in different population to test definitive role of vitamin D with heart failure and showed significant link of vitamin D deficiencies with heart failure.<sup>17</sup> Corroborating with earlier reports, in the present study also we observed diminished plasma 25-OH vitamin D<sub>3</sub> levels in heart failure patients compared to healthy controls. In addition, we grouped all subjects into three categories (a) sufficient (>30 ng/mL), (b) insufficient (10-20 ng/mL), (c) deficient (<10 ng/mL) and investigated prevalence in controls and patients. 25-OH vitamin D<sub>3</sub> deficient were highly frequent in heart failure patients (28%) compared to controls (15%). A recent meta-analysis on outcome of vitamin D supplementation in heart failure patients demonstrated suppression of parathyroid hormone and various inflammatory molecules.<sup>10</sup> However, clinical trials on supplementation of vitamin D in Chinese patients are lacking, and thus, double-blind vitamin D supplementation studies are required in Chinese patients to conclude therapeutic potential of vitamin D against heart failure.

Role of vitamin D in heart failure has been demonstrated elegantly. Lower levels of vitamin D stimulate renin-angiotensin-aldosterone

system and elevate vascular tone by release of angiotensin-II and lead to development of hypertension. In addition, majority of vitamin D supplementation studies revealed beneficial role of vitamin D against arterial stiffness.<sup>18</sup> Furthermore, administration of vitamin D suppresses activity of matrix metalloproteinase, an important molecule for pathogenesis of heart failure. Vitamin D also diminish inflammation through various pathways, and various studies have demonstrated protective phenomenon of vitamin D against development of atherosclerosis.<sup>19</sup>

Investigations on association between VDR polymorphisms and heart failure are limited. In American population, VDR variants failed to demonstrate possible association with susceptibility/resistance to heart failure.<sup>20</sup> Chronic heart failure patients with VDR FF genotype had higher rates of bone loss compared to other genotypes in Japanese patients.<sup>21</sup> In the present study, we observed significant association of FokI and TaqI heterozygous and minor allele with susceptibility to development of heart failure. In contrast, distribution of the other two common polymorphisms (BsmI and Apal) was comparable, indicating noninvolvement of these variants on pathogenesis of heart failure. The mechanism how FokI and TaqI variants linked with heart failure is not known. It is believed that FokI and TaqI variants affect vitamin D responses,<sup>22</sup> and this could be possible reason for susceptibility to heart failure.

As vitamin D-related response dependent on levels of vitamin D and its receptor, combined analysis of 25-OH vitamin D<sub>3</sub> and VDR polymorphisms are essential to decipher actual role of vitamin D molecules in heart failure. A recent study investigated combined analysis of VDR variants and levels of 25-OH vitamin D<sub>3</sub> and demonstrated a significant role of VDR and plasma levels with susceptibility to SLE and lupus nephritis.<sup>11</sup> In the present study also, we observed a significant combined role of VDR and plasma vitamin D with susceptibility to heart failure; subjects with VDR mutants and insufficient/deficient 25-OH vitamin D<sub>3</sub> levels are likely to have heart failure compared to other combination. Combinedly these observations suggest that both VDR variants and plasma levels of vitamin D<sub>3</sub> are essential for vitamin D<sub>3</sub> linked signaling phenotype and indicating individual-specific vitamin D supplementation strategy would be beneficial for management of heart failure.

Although our study is first of its kind to investigate vitamin D status and VDR genetic variants together, the present study has several limitations. First, there are several factors responsible for plasma levels of vitamin D such as extent of sun exposure, season, time of day, clothing style, pigmentation of skin, use of sunscreen,<sup>23</sup> and these factors were not considered for analysis in our study. Secondly, we have not adjusted various risk factors responsible for alteration of vitamin D levels and possibly associated with disease pathogenesis. Third, other genes responsible for vitamin D synthesis were not investigated in the present study.

In conclusion, heart failure patients display lower 25-OH vitamin D<sub>3</sub> levels compared to healthy controls. Common VDR variants (FokI and TaqI) are associated with susceptibility to development of heart failure. Combined analysis revealed importance of both plasma 25-OH vitamin D<sub>3</sub> levels and VDR polymorphism on determination of

HF predisposition. However, further studies with larger samples size in different populations are required to validate our findings.

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