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Genetic variation in *CYP27B1* is associated with congestive heart failure in patients with hypertension

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Abstract

Aims—We tested the hypothesis that genetic variation in vitamin D-dependent signaling is associated with congestive heart failure in human subjects with hypertension.

Materials & methods—Functional polymorphisms were selected from five candidate genes: *CYP27B1*, *CYP24A1*, *VDR*, *REN* and *ACE*. Using the Marshfield Clinic Personalized Medicine Research Project, we genotyped 205 subjects with hypertension and congestive heart failure, 206 subjects with hypertension alone and 206 controls (frequency matched by age and gender).

Results—In the context of hypertension, a SNP in *CYP27B1* was associated with congestive heart failure (odds ratio: 2.14 for subjects homozygous for the C allele; 95% CI: 1.05–4.39).

Conclusion—Genetic variation in vitamin D biosynthesis is associated with increased risk of heart failure.

Keywords

congestive heart failure; genetics; hypertension; vitamin D

Calcitropic hormones like 1,25-dihydroxy vitamin D₃ (1,25[OH]₂D₃) have long been known to influence blood pressure [1]. Altered myocardial function has been demonstrated in the presence of 1,25(OH)₂D₃ deficiency [2–4]. These relationships are partly independent of signaling factors that mediate the downstream hormonal manifestations of 1,25(OH)₂D₃ (e.g., serum calcium) [5].

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Hydroxylation by cytochrome P450 27B1 (CYP27B1) is the rate-limiting step in the activation of vitamin D and production of $1,25(\text{OH})_2\text{D}_3$ [6,7]. Another hydroxylase, CYP24A1, represents the first step in the inactivation of $1,25(\text{OH})_2\text{D}_3$ [8,9]. The activated hormone $1,25(\text{OH})_2\text{D}_3$ binds to a nuclear vitamin D receptor (VDR) [10] and, through heterodimeric interactions (e.g., with the retinoid X receptor), liganded VDR can function as a potent negative regulator of renin gene transcription [11]. VDR is expressed within a variety of cell types and cardiovascular tissues, including ventricular myocytes [12–15].

We have previously demonstrated that cardiomyocytes contain receptors for $1,25(\text{OH})_2\text{D}_3$ [11,16–20]. Animal models of congestive heart failure (CHF), developed in our laboratory, reveal large increases in left ventricular pressure in the context of $1,25(\text{OH})_2\text{D}_3$ deficiency [21]. These changes are the result of remodeling within the left ventricle and are direct [14, 22].

It has long been recognized that the resulting left ventricular hypertrophy (LVH) cannot be reversed by maintenance of normal serum calcium and phosphate levels, nor can it be reversed through the normalization of blood pressure [23]. Collectively, these studies indicate that perturbations in vitamin D homeostasis influence development of LVH and CHF in the context of hypertension (HTN).

We therefore hypothesized that genetic variability in the production, degradation, and ligand-dependent signaling of this hormone would alter the course of hypertensive heart disease. To test this hypothesis, we characterized the frequency of known functional polymorphisms in five pivotal candidate genes regulating this physiologic network, and quantified their association with clinical phenotypic traits corresponding to hypertensive heart disease, using one of the largest population-based DNA biobanks in the USA [24,25].

Materials & methods

Study population

All subjects for this study were participants in the Personalized Medicine Research Project (PMRP) [24,25]. This biobank is linked to coded clinical data from a patient cohort located in Central and Northern Wisconsin, USA. The target population for this biobank resides within the Central Marshfield Epidemiological Study Area (MESA), a geographically-based region of 19 zip codes surrounding the city of Marshfield (WI, USA). The region contains a relatively homogeneous and stable, trackable population, largely of European ancestry, who experience a pattern of disease similar to the distribution across Midwestern USA.

The project was approved by the Marshfield Clinic Institutional Review Board and all subjects gave written informed consent to participate in PMRP.

Phenotyping

These data were extracted from the Marshfield Clinic data warehouse, an extensive archive of medical information from all Marshfield Clinic facilities and affiliated hospitals, including: a full electronic medical chart; several enhanced clinical registries; reports of procedures, imaging studies, clinical laboratory data, and a file of more than 124 million patient diagnoses. Because of the stability of the population (low in- and out-migration rates), the high capture rate for inpatient and outpatient events (98 and 95%, respectively), and the longitudinal nature of the data (in most cases, spanning one to two decades), this record is considered comprehensive.

Medical records for all PMRP participants were interrogated electronically for relevant diagnostic codes and relevant procedural codes. A two-tiered strategy was employed to identify

subjects for the current study. First, subjects with HTN and CHF were flagged through the use of diagnostic codes from the 'International Classification of Diseases, Ninth Revision (ICD-9), Clinical Modification' (HTN: 401.0–405.99, CHF: 428.0–428.9). Patients with secondary HTN were excluded, based upon the presence of diagnostic codes for hyperaldosteronism, pheochromocytoma, renovascular disease, chronic renal failure, Cushing's syndrome, sleep apnea and coarctation of the aorta. Second, the medical records for all PMRP participants were also screened to flag each unique subject who had undergone transthoracic echocardiography. From the records of nearly 20,000 database participants, application of these criteria (requiring the presence of both echocardiographic data and relevant diagnostic codes) identified 205 hypertensive subjects with CHF (and corresponding echocardiographic data), 1171 hypertensive subjects without CHF (and corresponding echocardiographic data). From all subjects within the entire PMRP database having available echocardiographic data, we also identified 1383 additional study subjects without HTN and without CHF; this latter group served as a final selection pool for controls without either disease.

Using the 205 hypertensive subjects with CHF (and corresponding echocardiographic data) as our primary case group (205 HTN⁺/CHF⁺), we then identified a similar number of age- and gender-based frequency matched hypertensive subjects nested within the 1171 hypertensive subjects without CHF (206 HTN⁺/CHF⁻), and a similar number of frequency matched subjects without HTN from the 1383 potential controls (206 HTN⁻/CHF⁻). Our final study cohort therefore consisted of 617 subjects. Echocardiographic data for each of these subjects were then manually abstracted and entered into our final database.

Genotyping

Five candidate genes were selected for their role in the regulation of the vitamin D axis: *CYP27B1* (the rate limiting enzyme in vitamin D biosynthesis), *CYP24A1* (the first enzymatic step in vitamin D degradation), *VDR* (the vitamin D receptor), *REN* (renin), and *ACE* (angiotensin-converting enzyme). The latter two factors were selected because the expression of renin is known to be modulated by the activated VDR [11], and because the renin–angiotensin–aldosterone axis has been implicated in left ventricular remodeling [26].

A screening set of polymorphisms was then selected from existing databases (e.g., dbSNP) for the candidate genes represented in Table 1. Each variant was selected according to two criteria: first, high average heterozygosity (>10%) within subjects of European heritage (the population served by Marshfield Clinic is 98% Caucasian); and second, previously documented association with cardiometabolic risk determinants. For example, rs4646536 in *CYP27B1*, minor allele frequency 0.37 in Europeans, has previously been associated with Type 1 diabetes mellitus [27].

All genotyping was performed by laboratory personnel who were blinded to phenotypic classification. Our approach utilized a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) system. This strategy employs amplification involving a primer extension step, and the primer extension product is determined on a mass spectrometer. To design primers, a sequence encompassing each polymorphism was entered into proprietary software (SpectroDESIGNER) provided by Sequenom[®], Inc. (CA, USA). This software selects amplification and extension primers that can be multiplexed.

For each study subject, 15 nl of previously extracted DNA [24] was transferred from a 384-microtiter plate and spotted onto the pad of the 384-SpectroCHIP[®] bioarray (Sequenom, Inc.). Chip spotting is carried out using the SpectroPOINT instrument (Sequenom, Inc.), which is a 24 head pin tool system having the ability to spot a 384 element chip (SpectroCHIP) within 12–15 min. The SpectroCHIP was placed into the MALDI-TOF, and the mass and correlating

genotype were determined in real time using the MassARRAY® reverse transcription (RT) software (Sequenom, Inc.).

Statistical analysis

Clinical characteristics were compared for all study participants (205 HTN⁺/CHF⁺ cases, 206 HTN⁺/CHF⁻ cases, and 206 HTN⁻/CHF⁻ controls) using χ^2 test for categorical variables and analysis of variance for continuous variables. Nonparametric analysis of variance (Kruskal–Wallis test) was used to assess the differences in left ventricular mass index between groups. Genotypes and allele frequencies were compared using χ^2 and Fisher's exact tests. The odds ratios and 95% CI were estimated by logistic regression, adjusting for age, gender, BMI, smoking status, family history of HTN or CHF, and left ventricular mass index (LVMI), variables shown to be significantly different between cases and controls where there were sufficient numbers to include them in the multivariate models. LVMI was calculated in SAS 9.1 (SAS Institute, NC, USA), using routine clinical echocardiographic data obtained through M-mode methods: LV mass = $0.80 \times 1.04 ([\text{interventricular septum (IVS)} + \text{left ventricular end diastolic (LVED)} \text{ diameter} + \text{posterior wall thickness (PWT)}]^3 - [\text{LVED}]^3) + 0.6$. SAS 9.1 was used for all statistical analyses. p-values of less than 0.05 were considered to be statistically significant.

Results

The clinical characteristics for all study subjects are summarized in Table 2, at the time of most recent echocardiogram. As noted, subjects were frequency matched according to age and gender. Furthermore, hypertensive subjects with and without CHF were frequency matched according to their duration of HTN. The study population was predominantly Caucasian, which matches the ethnic composition of the community (98% of PMRP participants report Northern European ancestry [24]). As anticipated, the left ventricular mass index was higher ($115.3 \pm 36.1 \text{ g/m}^2$) in the HTN⁺/CHF⁺ group than in the HTN⁺/CHF⁻ group ($101.5 \pm 24.0 \text{ g/m}^2$) and the HTN⁻/CHF⁻ control group ($88.7 \pm 24.8 \text{ g/m}^2$). Similarly, the left ventricular ejection fraction (LVEF) was lowest, frequency of dilated cardiomyopathy (based upon diagnostic codes) was greatest, and devices such as implantable defibrillators and pacemakers were more common, within the HTN⁺/CHF⁺ groups (versus either the HTN⁺/CHF⁻ group or the HTN⁻/CHF⁻ controls).

Allele frequencies are shown in Table 3 for the 13 SNPs genotyped in this study. For each polymorphism, genotype frequency was compared between hypertensive subjects and hypertensive subjects with CHF (primary analysis). Genotype frequency was also compared between the initial group (i.e., hypertensive subjects without CHF) and the nonhypertensive controls (secondary analysis). Hence, the odds ratio was calculated twice. As shown in Table 3, logistic regression revealed two significant associations across groups: one in the primary analysis (rs4646536 for HTN⁺/CHF⁺ vs HTN⁺/CHF⁻); and one in the secondary analysis (rs4646537 for HTN⁺/CHF⁻ vs HTN⁻/CHF⁻). Although the possibility exists that these SNPs were associated by chance (i.e., since two hypotheses were tested repeatedly for 13 separate SNPs), the results remain significant after Bonferroni correction ($p < 0.05$) and it should be noted that both SNPs occur within the same gene, *CYP27B1*. Furthermore, the specific variant associated with CHF (rs4646536) has recently been shown to alter circulating levels of 25-OH vitamin D [28].

For rs4646536 (a SNP in the sixth intron of *CYP27B1*), the homozygous CC genotype was present at a frequency of 12.7% in the HTN⁺/CHF⁺ subjects, and a frequency of 8.7% in the HTN⁺/CHF⁻ subjects. Homozygosity for the minor allele at rs4646536 was therefore associated with an increased risk for CHF in patients with HTN (odds ratio [OR]: 2.14, 95% CI: 1.05–4.39; $p < 0.05$). We therefore also abstracted the most recent echocardiogram for each

of these subjects ($n = 205$ HTN⁺/CHF⁺ subjects, and $n = 206$ HTN⁺/CHF⁻ subjects), and we tested the echocardiographic parameters for association with genotype at rs4646536. As shown in Table 4, these parameters included left atrial (LA) area, IVS thickness, left ventricular end systolic (LVES) dimension, LVED dimension, ejection fraction (EF) and posterolateral ventricular (PLV) wall thickness. None of these parameters differed according to rs4646536 genotype. A possible explanation for the absence of statistical significance is the low numbers of patients (21 controls, 18 HTN and 26 HTN and CHF) with the low abundance CC genotype. An alternative explanation might be the lack of uniformly ascertained echocardiographic data (the current data were abstracted from clinical records).

No other candidate gene polymorphism within the current study was associated with altered risk for CHF in the context of HTN (Table 3). Lack of association for markers in candidate genes beyond *CYP27B1* should not be interpreted as conclusive evidence that the other genes are not important in the development and progression of CHF. A more comprehensive analysis of the linkage structure within each gene would be required before such a statement could be made.

Lastly, we made an effort to determine if any of the polymorphisms characterized within the current study are found more frequently in patients with HTN. This second analysis (HTN⁺/CHF⁻ vs HTN⁻/CHF⁻) revealed that an additional SNP in *CYP27B1* was associated with altered risk for the development of HTN. For rs4646537 (a SNP in the eighth intron of *CYP27B1*), carrier status for the minor allele (heterozygous AC genotype) demonstrated protective effect against development of HTN (OR: 0.35, 95% CI: 0.13–0.91, $p < 0.05$). The heterozygous genotype was noted in 3.9% of HTN⁺/CHF⁻ subjects, and in 10.2% of the HTN⁻/CHF⁻ controls. No subjects were homozygous for the minor allele at rs4646537. Furthermore, the mean systolic blood pressure of control subjects was 144 mmHg in this study (Table 2), suggesting that many of the controls may have had mild (undiagnosed) HTN. Thus, lack of association for markers in candidate genes beyond *CYP27B1* should not be interpreted as conclusive evidence that the other genes are also not important in the development of HTN.

Discussion

The current study demonstrates that a common SNP in *CYP27B1* (rs4646536) is associated with CHF in patients with HTN. The gene product for *CYP27B1* (25-OH vitamin D 1 α hydroxylase) is the rate-limiting enzyme for bioactivation of 1,25(OH)₂ vitamin D, and it is well known that vitamin D insufficiency can influence the severity and progression of CHF [29–32]. Observations made in our current study therefore suggest that genetic variability in vitamin D homeostasis may also contribute to the pathogenesis of CHF.

The mechanism linking rs4646536 to CHF remains unclear. This SNP represents one of 12 SNPs recorded for the *CYP27B1* gene in dbSNP Build 129. Since all 12 *CYP27B1* SNPs are located within a single block of linkage disequilibrium, it is conceivable that rs4646536 tags an as yet unrecognized causative allele. It is also conceivable that rs4646536 (which is located in the sixth intron of the *CYP27B1* gene) disrupts a critical regulatory function within this gene. Genetic variability in the metabolism of 25(OH)D could certainly then alter the role of 25(OH)D and 1,25(OH)₂D within metabolic feedback loops regulating the tone of this hormonal axis [33]. Mutations that inactivate *CYP27B1* have long been known to reduce 1,25(OH)₂D levels [34].

It is noteworthy that rs4646537, a second intronic SNP in this same gene (i.e., within the same block of linkage disequilibrium) was associated with decreased patient risk for the development of HTN. By activating the nuclear VDR, 1,25(OH)₂D₃ functions as a regulator of renin gene expression [23,27]. We report that a variant (rs4646536) of *CYP27B1* is associated with

increased heart failure phenotype and another variant (rs4646537) seemingly protects from HTN. Further studies are required to determine if these genotypes and phenotypes relate to circulating vitamin D metabolite levels. Importantly, ablation of CYP27B1 in knockout mice has recently been shown to cause a hypertensive phenotype with increased tone in the renin–angiotensin system [5]. It is well known that functional alteration in the renin–angiotensin–aldosterone axis can have profound impact on blood pressure [26]. Thus, variability in vitamin D-dependent modulation of the renin–angiotensin–aldosterone axis could play a role in the development of HTN, independent from its influence on CHF (i.e., hypertensive heart disease).

Animal studies conducted in our laboratory have clearly demonstrated that vitamin D deficiency alters myocardial function and morphology along with an alteration in the composition of the extracellular matrix [22,23]. Moreover, the expression of metalloproteases MMP-2 and MMP-9 are upregulated in genetically modified VDR^{-/-} mice [35]. We and others have also shown that genetically modified VDR^{-/-} mice develop cardiac hypertrophy and myocardial fibrosis accompanied by increased interstitial collagen deposition [21,33]. Therefore, it seems likely that similar perturbations would be associated with left ventricular hypertrophy in humans. However, echocardiographic data from the current study do not solidly support this claim.

We observed no clear association between the intronic SNP linked to CHF diagnostic codes (rs4646536) and a number of clinically relevant echocardiographic end points. This may be due to the low number of patients in our study who were homozygous for the minor allele, and/or it may be due to clinical heterogeneity of the patient population with respect to severity of CHF. Our echocardiographic data were abstracted from clinical records. It is likely that there were multiple technicians obtaining the data, and multiple readers interpreting the results. Hence, additional longitudinal studies are needed, with rigorous ascertainment of echocardiographic data, to clarify the impact of rs4646536 on the rate of progression for hypertensive heart disease.

Lastly, an alternative mechanism (e.g., changes in myocardial function not evident on routine echocardiography) may underlie our observed association between human genetic variation in *CYP27B1* and CHF in patients with HTN. Animal studies reveal that 1,25(OH)₂D₃ affects numerous cellular processes central to the function of cardiomyocytes. For example, 1,25(OH)₂D₃ has been shown to increase the sensitivity of the heart to contractile stimuli [14]. Green *et al.* have shown that 1,25(OH)₂D₃ affects the intracellular Ca²⁺ transient, and shortening, in fura-2-loaded adult cardiac myocytes [36]. They observed that changes in the Ca²⁺ transient contributed, in part, to the decrease in contraction peak shortening and accelerated relaxation. Whether such a relationship contributes to subtle inotropic (or subtle chronotropic) changes predisposing hypertensive patients with rs4646536 to CHF remains to be determined.

Conclusion

This study demonstrates for the first time that genetic variability in *CYP27B1* may influence the development of CHF in human subjects with HTN. Confirmatory studies will be needed, including populations of diverse ancestry.

Executive summary

- Vitamin D insufficiency is highly prevalent within the general community.
- Vitamin D insufficiency has been associated with congestive heart failure.
- rs4646536 and rs4646537 are intronic SNPs within the *CYP27B1* gene.

- CYP27B1 is the rate-limiting step in the biosynthesis of 1,25(OH)₂ vitamin D.

Results

- rs4646536 is associated with congestive heart failure in patients with hypertension.
- rs4646537 is associated with hypertension.

Discussion

- Genetic variability in the biosynthesis of 1,25(OH)₂ vitamin D alters risk of heart disease.
- Large confirmatory studies are needed to quantify the public health impact of this finding.

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•• of considerable interest

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Table 1

Markers genotyped in the current study.

Gene	Polymorphism	Alleles
<i>CYP27B1</i>	rs4646537	A/C
<i>CYP27B1</i>	rs4646536	T/C
<i>CYP24A1</i>	rs912505	A/G
<i>CYP24A1</i>	rs6068816	C/T
<i>CYP24A1</i>	rs4809960	T/C
<i>VDR</i>	rs1544410	G/A
<i>VDR</i>	rs7975232	C/A
<i>VDR</i>	rs731236	C/T
<i>VDR</i>	rs10735810	G/A
Renin	rs10900555	T/C
Renin	rs2272237	C/G
Renin	rs2887284	C/A
<i>ACE</i>	rs4646994	A/T

ACE: Angiotensin-converting enzyme; VDR: Vitamin D receptor.

Table 2

Clinical characteristics at the time of most recent echocardiogram.

Characteristics	Controls (n = 206)	HTN (n = 206)	CHF + HTN (n = 205)	p-value
Age (mean $\pm$ SD)	58.5 $\pm$ 20.5	59.4 $\pm$ 13.8	60.2 $\pm$ 13.0	0.59
Male (distribution)	101 (49.0%)	101 (49.0%)	101 (49.3%)	1.00
Female (distribution)	105 (51.0%)	105 (51.0%)	104 (50.7%)	
Caucasian (distribution)	167 (81.5%)	181 (87.9%)	171 (83.8%)	0.11
Other race (distribution)	5 (2.4%)	–	2 (1.0%)	
Both races (distribution)	33 (16.1%)	25 (12.1%)	21 (15.2%)	
HTN in FMH (distribution)	56 (30.1%)	128 (67.7%)	130 (69.5%)	<0.001
CHF in FMH (distribution)	13 (6.9%)	28 (15.3%)	52 (28.9%)	<0.001
Systolic blood pressure (mean $\pm$ SD)	144.5 $\pm$ 16.6	170.0 $\pm$ 17.7	166.5 $\pm$ 18.1	<0.001
Diastolic blood pressure (mean $\pm$ SD)	78.0 $\pm$ 10.5	81.7 $\pm$ 14.4	79.4 $\pm$ 12.9	0.01
Heart rate (BPM, mean $\pm$ SD)	70.4 $\pm$ 14.1	67.5 $\pm$ 11.1	77.3 $\pm$ 21.2	0.009
BMI (kg/m ² , mean $\pm$ SD)	27.4 $\pm$ 4.9	29.1 $\pm$ 4.9	30.5 $\pm$ 6.0	<0.001
LV mass index (mean $\pm$ SD)	88.7 $\pm$ 24.8	101.5 $\pm$ 24.0	115.3 $\pm$ 36.1	<0.001
LVEF (mean $\pm$ SD)	0.61 $\pm$ 0.09	0.59 $\pm$ 0.10	0.51 $\pm$ 0.13	<0.001
Ever smoked (distribution)	82 (39.8%)	101 (49.3%)	119 (58.1%)	0.001
Dilated CM (distribution)	3 (1.5%)	14 (6.8%)	70 (34.2%)	<0.001
Implantable defibrillator or pacemaker (distribution)	5 (2.4%)	6 (2.9%)	36 (17.6%)	<0.001

BPM: Beats per minute; CHF: Congestive heart failure; CM: Cardiomyopathy; FMH: Fibromuscular hyperplasia; HTN: Hypertension; LV: Left ventricle; LVEF: Left ventricular ejection fraction; SD: Standard deviation.

Table 3

Logistic regression analysis adjusting for age, gender, BMI, smoking status, left ventricular mass index and family history of hypertension or congestive heart failure.

Genotype	Controls (%) (n = 206)	Hypertension		Congestive heart failure and hypertension	
		Subjects (%) (n = 206)	Odds ratio* (95% CI)	Subjects (%) (n = 205)	Odds ratio* (95% CI)
rs4646536 (CYP27B1)					
TT	91 (44.2%)	104 (50.5%)	1.00	93 (45.4%)	1.00
CT	94 (45.6%)	84 (40.8%)	1.06 (0.65–1.72)	86 (41.9%)	1.09 (0.63–1.89)
CC	21 (10.2%)	18 (8.7%)	0.86 (0.39–1.92)	26 (12.7%)	1.48 (0.66–3.30)
CC+CT			1.02 (0.64–1.61)		1.17 (0.70–1.96)
rs4646537 (CYP27B1)					
AA	185 (89.8%)	198 (96.1%)	1.00	192 (93.7%)	1.00
AC	21 (10.2%)	8 (3.9%)	0.35 (0.13–0.91)	12 (5.9%)	0.53 (0.20–1.40)
CC	–	–	–	1 (0.5%)	N/A
rs4809960 (CYP24A1)					
TT	127 (61.7%)	123 (59.7%)	1.00	118 (57.6%)	1.00
CT	70 (34.0%)	72 (35.0%)	0.97 (0.59–1.58)	76 (37.1%)	1.13 (0.66–1.94)
CC	9 (4.4%)	11 (5.3%)	1.02 (0.33–3.13)	11 (5.4%)	0.88 (0.24–3.29)
CC+CT			0.97 (0.61–1.56)		1.10 (0.66–1.86)
rs6068816 (CYP24A1)					
CC	167 (81.1%)	167 (81.1%)	1.00	174 (84.9%)	1.00
CT	38 (18.4%)	39 (18.9%)	1.34 (0.74–2.44)	29 (14.1%)	1.00 (0.52–1.95)
TT	1 (0.5%)	–	N/A	2 (1.0%)	N/A
rs912505 (CYP24A1)					
AA	138 (67.0%)	136 (66.0%)	1.00	131 (63.9%)	1.00
AG	60 (29.1%)	63 (30.6%)	0.85 (0.51–1.41)	66 (32.2%)	1.11 (0.65–1.92)
GG	8 (3.9%)	7 (3.4%)	0.90 (0.25–3.29)	8 (3.9%)	0.94 (0.23–3.78)
GG+AG			0.85 (0.52–1.39)		1.10 (0.65–1.86)
rs10735810 (VDR)					
GG	88 (42.7%)	74 (35.9%)	1.00	79 (38.5%)	1.00

Genotype	Controls (%) (n = 206)	Hypertension		Congestive heart failure and hypertension		
		Subjects (%) (n = 206)	Odds ratio* (95% CI)	Subjects (%) (n = 205)	Odds ratio* (95% CI)	Odds ratio [‡] (95% CI)
AG	90 (43.7%)	96 (46.6%)	1.09 (0.66–1.81)	97 (47.3%)	0.82 (0.47–1.42)	0.82 (0.51–1.32)
AA	28 (13.6%)	36 (17.5%)	1.33 (0.67–2.62)	29 (14.2%)	0.85 (0.39–1.88)	0.70 (0.37–1.33)
AA+AG			1.15 (0.71–1.84)		0.82 (0.49–1.39)	0.79 (0.50–1.23)
rs1544410 (VDR)						
GG	70 (34.0%)	74 (35.9%)	1.00	65 (31.7%)	1.00	1.00
AG	106 (51.4%)	105 (51.0%)	1.05 (0.64–1.73)	101 (49.3%)	1.04 (0.59–1.81)	1.04 (0.64–1.67)
AA	30 (14.6%)	27 (13.1%)	1.10 (0.53–2.30)	39 (19.0%)	1.51 (0.69–3.32)	1.42 (0.73–2.74)
AA+AG			1.06 (0.66–1.71)		1.13 (0.66–1.92)	1.12 (0.71–1.76)
rs7975232 (VDR)						
CC	43 (20.9%)	40 (19.4%)	1.00	38 (18.5%)	1.00	1.00
AC	114 (55.3%)	115 (55.8%)	1.37 (0.75–2.49)	100 (48.8%)	1.11 (0.58–2.13)	0.73 (0.41–1.30)
AA	49 (23.8%)	51 (24.8%)	1.46 (0.73–2.91)	67 (32.7%)	1.29 (0.62–2.67)	0.96 (0.51–1.83)
AA+AC			1.40 (0.79–2.48)		1.17 (0.63–2.16)	0.80 (0.46–1.40)
rs731236 (VDR)						
TT	71 (34.5%)	77 (37.4%)	1.00	67 (32.7%)	1.00	1.00
CT	107 (51.9%)	103 (50.0%)	0.94 (0.57–1.54)	101 (49.3%)	0.91 (0.52–1.59)	1.00 (0.62–1.61)
CC	28 (13.6%)	26 (12.6%)	1.12 (0.52–2.39)	37 (18.1%)	1.61 (0.72–3.60)	1.37 (0.70–2.67)
CC+CT			0.97 (0.60–1.56)		1.03 (0.61–1.75)	1.08 (0.68–1.69)
rs10900555 (REN)						
TT	89 (43.2%)	83 (40.3%)	1.00	80 (39.0%)	1.00	1.00
CT	95 (46.1%)	93 (45.2%)	0.92 (0.56–1.51)	102 (49.8%)	1.26 (0.73–2.16)	1.07 (0.67–1.70)
CC	22 (10.7%)	30 (14.6%)	1.30 (0.63–2.71)	23 (11.2%)	0.91 (0.38–2.17)	0.71 (0.35–1.42)
CC+CT			1.00 (0.63–1.59)		1.18 (0.71–1.98)	0.98 (0.63–1.52)
rs2272237 (REN)						
CC	88 (42.7%)	83 (40.3%)	1.00	82 (40.0%)	1.00	1.00
CG	90 (43.7%)	100 (48.5%)	1.25 (0.76–2.04)	95 (46.3%)	1.28 (0.74–2.20)	0.97 (0.61–1.54)
GG	28 (13.6%)	23 (11.2%)	0.91 (0.43–1.90)	28 (13.7%)	1.06 (0.48–2.37)	1.12 (0.55–2.29)
GG+CG			1.16 (0.73–1.86)		1.22 (0.73–2.04)	1.00 (0.64–1.55)

Genotype	Controls (%) (n = 206)	Hypertension		Congestive heart failure and hypertension	
		Subjects (%) (n = 206)	Odds ratio* (95% CI)	Subjects (%) (n = 205)	Odds ratio* (95% CI)
rs2887284 (REN)					
CC	128 (62.1%)	124 (60.2%)	1.00	127 (61.9%)	1.00
AC	66 (32.0%)	77 (37.4%)	1.35 (0.83–2.20)	68 (33.2%)	1.42 (0.82–2.48)
AA	12 (5.8%)	5 (2.4%)	0.40 (0.09–1.68)	10 (4.9%)	0.75 (0.20–2.83)
AA+AC			1.21 (0.76–1.94)		1.32 (0.78–2.25)
rs4646994 (ACE)					
AA	56 (27.2%)	55 (26.7%)	1.00	57 (27.8%)	1.00
AT	95 (46.1%)	103 (50.0%)	1.11 (0.64–1.93)	97 (47.3%)	1.16 (0.63–2.14)
TT	55 (26.7%)	48 (23.3%)	0.97 (0.52–1.80)	51 (24.9%)	0.99 (0.50–1.98)
TT+AT			1.05 (0.63–1.76)		1.10 (0.62–1.93)

Bolding indicates statistical significance at a p-value <0.05.

* Compared with nonhypertensive controls.

‡ Compared with hypertensive subjects.

N/A: Not applicable; VDR: Vitamin D receptor.

Table 4

Hypertensive subjects with and without diagnostic codes for congestive heart failure.

Parameter	CC n = 26	CT n = 86	TT n = 93	p-value
<i>Association of rs4646536 versus echo variables (n = 205) with congestive heart failure</i>				
LA area (cm ²)	4.47 ± 1.25	4.15 ± 0.81	4.20 ± 0.85	0.28
IVS thickness	1.14 ± 0.25	1.18 ± 0.21	1.14 ± 0.18	0.36
LVES dimension	3.62 ± 1.31	3.22 ± 0.81	3.37 ± 1.04	0.19
LVED dimension	5.02 ± 1.00	4.88 ± 0.76	4.93 ± 0.91	0.76
Ejection fraction	51.4 ± 16.8	53.7 ± 14.5	53.4 ± 14.3	0.81
PLV wall thickness	1.12 ± 0.18	1.14 ± 0.16	1.11 ± 0.19	0.60
<i>Association of rs4646536 versus echo variables (n = 206) without congestive heart failure</i>				
LA area (cm ²)	3.52 ± 0.47	3.71 ± 0.60	3.63 ± 0.69	0.42
IVS thickness	1.01 ± 0.14	1.09 ± 0.16	1.08 ± 0.17	0.15
LVES dimension	2.84 ± 0.68	2.91 ± 0.67	2.90 ± 0.69	0.91
LVED dimension	4.40 ± 0.64	4.66 ± 0.63	4.65 ± 0.56	0.22
Ejection fraction	61.8 ± 10.0	59.8 ± 9.8	60.9 ± 9.3	0.69
PLV wall thickness	1.01 ± 0.13	1.07 ± 0.14	1.04 ± 0.14	0.18

IVS: Interventricular septal; LA: Left atrial; LVED: Left ventricular end diastolic; LVES: Left ventricular end systolic; PLV: Posterolateral ventricular.