Vitamin D gene polymorphisms and risk of acute cardiovascular events

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ABSTRACT

Background: Low vitamin D levels have been associated with various diseases, including cardiovascular disease, although data are not consistent. Serum vitamin D levels depend on different synthesis steps which are controlled by gene cascade. This study investigated whether low serum levels of vitamin D and single nucleotide polymorphisms (SNPs) in genes involved in the vitamin D cascade (VDR, CYP2R1, CYP27B1, CYP24 and DBP) could be risk factors for acute coronary syndrome (ACS).

Materials and methods: 71 Patients with ACS and 443 healthy controls were genotyped for polymorphisms in genes of the vitamin D cascade. Clinical findings were correlated with serum levels of the vitamin D metabolites 1,25(OH)₂D₃ and 25(OH)D₃.

Results: 25(OH)D₃ and 1,25(OH)₂D₃ insufficiency was more common in ACS patients than in controls ($p < 0.05$). After adjustments for variables and propensity score the SNPs rs4646536 and rs2296241 within CYP24 and CYP27B1 are significantly associated with ACS ($p < 0.05$). CYP27B1 rs4646536 genotype TT is more frequently found in ACS patients compared to healthy controls, whereas genotype TC is found at higher frequency in controls (79% vs 44% and 42% vs 5%, respectively, $p < 0.01$). CYP24 rs2296241 SNP genotype GG occurred less frequently in ACS vs controls (7% vs 18%), while genotype AG was found more frequently in ACS (79% vs 57%, $p < 0.01$).

Conclusions: Lower vitamin D levels as well as polymorphisms within the CYP27B1 and CYP24 genes seem to promote ACS. These findings may offer potential for identification of patients at increased risk for cardiovascular incidents.

1. Introduction

Vitamin D exists in two main forms, vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Vitamin D2 is predominantly obtained from eukaryote sources, like fungi and yeast, whereas vitamin D3 is produced from 7-dehydrocholesterol upon exposure to ultraviolet B radiation¹. First, 7-dehydrocholesterol is converted into pre-vitamin D3 then a thermo-sensitive process isomerises pre-vitamin D3 to vitamin D3 that is delivered into the circulation where it binds to vitamin D binding protein (DBP) for systemic transport and delivery to target organs. In the liver, vitamin D3 is sequentially hydroxylated by cytochrome P450 enzymes, via 25-hydroxylase (CYP2R1) among others, into 25-hydroxyvitamin D (25(OH)D; calcidiol or calcifediol), the major storage and circulating form of vitamin D²; 25(OH)D is frequently measured as an index of vitamin D status. Subsequently, a second

hydroxyl group is added by 1 α -hydroxylase (CYP27B1) to convert 25(OH)D into the active metabolite 1,25-dihydroxyvitamin D (1,25(OH)₂D₃; calcitriol) in the kidney.³ 1,25(OH)₂D₃ binds to the nuclear vitamin D receptor (VDR) in target organs and forms a heterodimer complex with the retinoid X receptor (RXR), recruiting other transcriptional cofactors to regulate target gene transcription by interacting with vitamin D response elements (VDRE) in gene promoter regions. A decisive step in the metabolic process is degradation of 25(OH)D and 1,25(OH)₂D₃ by 24-hydroxylase (CYP24A1)⁴.

There is a growing body of evidence to suggest that gene polymorphisms within the vitamin D cascade (DBP, CYP2R1, CYP27B1, CYP24 and VDR) may play an important role in the risk of developing a variety of autoimmune and non-autoimmune diseases⁵ by modulating the vitamin D serum levels.⁶ In addition, epidemiological studies have linked low serum levels of 25-(OH)D, with a range of medical

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conditions, including cardiovascular diseases.^{7–12} In particular, low levels of vitamin D have been associated with acute myocardial infarction (MI).^{13,14} There even is evidence that lower vitamin D levels are linked to increased mortality.¹⁵ Although the role of 25(OH)D is controversial¹⁶, several large prospective studies have demonstrated that higher levels of 25(OH)D are associated with a lower risk of cardiovascular morbidity and mortality compared with low 25(OH)D status.^{8,17–21} There appears to be an inverse correlation between low serum levels of 25(OH)D and atherosclerotic calcification, which is an important cardiovascular risk factor.^{22–24} Data suggests that 25(OH)D has an important effect in cardiomyocytes, vascular smooth muscle cells and the vascular endothelium.²⁵

Daily vitamin D supplementation has been shown to improve some cardiovascular risk markers in healthy overweight and obese subjects.²⁶ However, the mechanisms by which vitamin D and its metabolites may influence the development of cardiovascular diseases, including MI, are not fully understood yet. It has been postulated that 25(OH)D and/or 1,25(OH)₂D₃ could act directly via VDRs in cardiac smooth muscle cells in the vessels or muscle of the heart, or indirectly by promoting calcium absorption in the gut.²⁷

Nevertheless, at present the role of vitamin D in cardiovascular diseases remains unclear.²⁸ Thus, studies failed to find an association between vitamin D and a reduction in mortality due to cardiovascular risk²⁹ or blood pressure³⁰ or they found only a modest association.¹¹

Given the controversial role of vitamin D in the pathogenesis of cardiovascular diseases, this study investigated whether low serum levels of vitamin D and polymorphisms in the vitamin D cascade (VDR, CYP2R1, CYP27B1, CYP24 and DBP) could be risk factors for acute coronary syndrome (ACS).

2. Materials and methods

2.1. Study design

This single center prospective surveillance study was conducted at the University Hospital of Frankfurt am Main between 2010 and 2012. The study protocol was approved by local ethics committee of Frankfurt University Hospital, Frankfurt am Main. All participants gave written informed consent prior to enrollment into the trial, which was conducted in accordance to the Declaration of Helsinki.

2.2. Participants

All patients who attended the Department of Cardiology (cardiology ward or intensive care unit) at the University Hospital of Frankfurt am Main were eligible for enrollment into the study. Inclusion criteria were ACS, as defined by the ESC/ACCF/AHA/WHF Third Universal Definition³¹ and no vitamin D supplementation. There was no randomization or balanced selection for age, gender, race, region, medication or co-morbidities. A control group comprising volunteer blood donors without a history of coronary artery disease who were not receiving supplementary vitamin D was also included. Medical records, including diagnostic and therapeutic procedures, were obtained for all enrolled ACS patients.

2.3. Genotyping

Genomic DNA was obtained from whole blood within the first 6 h of hospital admittance and extracted using the salting-out procedure as previously described³² and amplified using polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP) or real time polymerase chain reaction (PCR-real time) methods for detection of single nucleotide polymorphisms (SNPs) within DBP (rs4588, rs7041), CYP2R1 (rs10741657, rs12794714), CYP24 (rs927650, rs2248137, rs2296241), CYP27B1 (rs10877012, rs4646536) and VDR (rs7975232, rs731236, rs1544410, rs10735810) genes, as previously described.

Table 1A

Baseline characteristics of the ACS study group (n = 71).

Age (years)	64 ± 13
Male	55 (75%)
BMI (body mass index) kg/m ²	27 ± 5
STEMI	39
NSTEMI	32
Left ventricular ejection fraction (%)	44 ± 14
Cardiac arrest	5 (7%)
Cardiogenic shock	14 (20%)
Prior history of myocardial infarction	16 (23%)
NYHA functional class	3 ± 1
Smoking	35 (49%)
Smoking (pack years)	35 ± 18
Chronic kidney disease	23 (32%)
Chronic kidney disease (CKD stadium)	3 ± 1
Dialysis	3 (4%)
Cholesterol (mg/dl)	174 ± 41
Triglycerides (mg/dl)	132 ± 70
HDL (mg/dl)	43 ± 14
LDL (mg/dl)	105 ± 35
HDL/LDL	3 ± 7
Leucocytes (/nl)	10 ± 4
HbA1c (%Hemoglobin)	6 ± 1
NT-pro-BNP (pg/ml)	3711 ± 6588
Troponin (ng/ml)	2441 ± 3377
Creatinine (mg/dl)	1 ± 1
BUN (mg/dl)	46 ± 29
PAD (peripheral artery disease)	11 (16%)
CAD (coronary artery disease)	71 (100%)
Coronary 1 vessel disease	21 (30%)
Coronary 2 vessel disease	18 (25%)
Coronary 3 vessel disease	32 (45%)
Atrial fibrillation	8 (11%)
Ventricular tachycardia	3 (4%)
Stroke	5 (7%)
Death	5 (7%)
Arterial hypertension	64 (92%)
Hyperlipidemia	38 (54%)
Diabetes	18 (25%)
Insulin dependent diabetes	10 (14%)
Family history for cardiovascular disease	19 (27%)
ASS	71 (100%)
Clopidogrel	34 (48%)
Prasugrel	36 (51%)
Betablocker	68 (96%)
ACE inhibitor	63 (89%)
ATRB	6 (9%)
Hydrochlorothiazide	67 (94%)
Digitalis	13 (18%)
Amiodarone	5 (7%)
Spironolactone	4 (6%)
Eplerenone	11 (16%)
Statin	68 (96%)

Table 1B

Baseline characteristics of the healthy volunteers group (n = 443).

Age (years)	35 ± 15
Male	193 (44%)

(Table 4)^{33–37} To confirm the accuracy of the methods, random samples of the studied polymorphisms were genotyped twice, and showed a concordance of 98%.

2.4. Measurement of 25(OH)D and 1,25(OH)₂D₃

Plasma levels of 25(OH)D and 1,25(OH)₂D₃ were measured by radioimmunoassay (DiaSorin, Stillwater, Minnesota, USA and IDS, Frankfurt am Main, Germany, respectively). Levels of 25(OH)D were defined as insufficient if they were < 20 ng/mL (or < 50 nmol/L)³⁸.

Table 2

Logistic regression analysis between ACS and the polymorphisms within DBP (rs4588, rs7041), CYP2R1 (rs10741657, rs12794714), CYP24 (rs927650, rs2248137, rs2296241), CYP27B1 (rs10877012, rs4646536) and VDR (rs7975232, rs731236, rs1544410, rs10735810) genes adjusted for age, sex and 25 OH vitamin D levels and adjustments for propensity score.

Gene	SNPs	Odds Ratio	Std. Err	(95% CI)	unadjusted p value	p value adjusted for age, sex, 25 OH Vit D	p value adjusted for propensity score
DBP	rs4588	1.42	0.58	0.64–3.17	0.51	0.17	0.31
	rs7041	1.41	0.53	0.68–2.94	0.50	0.15	0.13
CYP2R1	rs10741657	1.83	0.63	0.94–3.58	0.17	0.32	0.55
	rs12794714	1.38	0.48	0.69–2.74	0.11	0.77	0.68
CYP24	rs927650	0.84	0.31	0.41–1.72	0.86	0.22	0.25
	rs2248137	0.87	0.32	0.42–1.79	0.45	0.72	0.52
	rs2296241	0.53	0.24	0.21–1.30	< 0.001	< 0.001	0.02
CYP27B1	rs10877012	3.76	1.86	1.42–9.99	< 0.001	0.57	0.77
	rs4646536	1.77	0.80	0.75–4.20	< 0.001	< 0.001	< 0.001
	rs7975232	1.41	0.59	0.62–3.19	0.02	0.30	0.35
VDR	rs731236	0.98	0.36	0.48–2.02	0.69	0.39	0.35
	rs1544410	0.59	0.21	0.30–1.18	0.07	0.96	0.93
	rs10735810	1.14	0.35	0.62–2.09	0.97	0.33	0.42

Table 3A

Distribution of the rs2296241 polymorphism within the CYP24 and rs4646536 within the CYP27B1 in patients with ACS and healthy controls.

CYP24 rs2296241			CYP27B1 rs4646536		
Controls	ACS		Controls	ACS	
n (%)	n (%)		n (%)	n (%)	
AG	249 (56.5)	58 (81.3)	TC	191 (43.3)	56 (78.7)
AA	114 (25.8)	7 (9.9)	CC	61 (13.9)	9 (12.4)
GG	78 (17.7)	6 (8.8)	TT	189 (42.7)	6 (9.0)
p < 0.01			p < 0.01		

Table 3B

25(OH)₂D serum levels were still lower in the ACS patients. Therefore the p value reflects more the already insufficiency than a true influence of the polymorphism on the vitamin D levels. Vitamin levels are given as mean values.

CYP24 rs2296241			CYP27B1 rs4646536		
Controls	ACS		Controls	ACS	
25(OH) ₂ D (ng/mL)					
AG	18.6	16.0	TC	17.7	16.9
AA	19.1	17.6	CC	15.8	14.5
GG	16.0	17.0	TT	19.5	13.2
p = 0.144			p = 0.142		

Plasma concentrations of 1,25(OH)₂D₃ were considered to be in the normal range if they were 20–25 pg/mL, according to the manufacturer's instructions.

2.5. Statistics

Deviations from Hardy–Weinberg equilibrium, differences in genotype and allele distributions between groups were evaluated by Chi-squared (χ^2) test (BIAS software, package 9.08; Epsilon, Weinheim, Germany). Continuous variables were compared by analysis of variance (ANOVA). Categorical comparisons were made using Chi-squared analysis. Propensity score and regression on propensity score analysis were calculated as previously reported.^{39,40} Statistical significance was defined as a p-value of < 0.05. Statistical analyses were performed using SPSS for Mac OS X version 21.0 and using The R Project for Statistical Computing.

3. Results

3.1. Patient characteristics

Of 514 patients screened, 71 (55 male [75%]) had ACS and were enrolled in the study (Table 1A). All participants were Caucasians. Mean patient age was 64 years for males and 68 years for females. Patients were divided into two groups: those with an acute ST elevation MI (n = 39; 29 male [74%]) and those with an acute non-ST elevation MI (n = 32; 24 male [75%]). Five patients (7%) experienced cardiac arrest and underwent successful cardiopulmonary resuscitation, 14 patients (20%) were in cardiogenic shock, and 16 patients (23%) had a history of prior MI. A total of 443 healthy controls (244 male [55%]) were included (Table 1B).

3.2. Genotype analysis

All samples were in Hardy–Weinberg equilibrium (p > 0.05) for each polymorphism. Thirteen different polymorphisms for the genes encoding DBP, CYP2R1, CYP27B1, CYP24A1 and VDR were studied.

After adjustments for variables and adjustments for propensity score the SNPs rs4646536 and rs2296241 within CYP24 and CYP27B1 genes are significantly associated with ACS (p < 0.05, Table 2). In this regard in CYP27B1 rs4646536 genotype TT is more frequently found in patients with ACS compared to healthy controls, whereas genotype TC is found at higher frequency in controls compared to ACS patients (79% vs 44% and 42% vs 5%, respectively, p < 0.01), see Table 3A and B. In addition to that CYP24 rs2296241 SNP genotype GG occurred less frequently in ACS patients vs controls (7% vs 18%), while genotype AG was found more frequently in ACS (79% vs 57%, p < 0.01).

There were no significant differences between ACS patients and controls for any polymorphisms in genes encoding DBP, CYP2R1 or VDR genes. There was also no significant difference in these genes when patients were stratified according to gender and age (data not shown). Due to the small sample size, haplotype analysis was not performed.

3.3. Plasma levels of 25(OH)D and 1,25(OH)₂D₃

25(OH)D insufficiency (< 20 ng/mL) was found in 81% of ACS patients compared with 28.6% of controls (Fig. 1A and B). When measured within 6 h of diagnosis, ACS patients had significantly lower 25(OH)D levels than controls (13.2 vs 21.3 ng/mL, p < 0.05).

Low levels of 1,25(OH)₂D₃ (< 20 pg/mL) were found in 78% of ACS patients and 14% of controls, and patients had significantly lower plasma levels compared with controls (21.5 vs 16.1 pg/mL, p < 0.05). Interestingly, patients carrying the genotype GG of the rs2248137

Table 4
Genotyping conditions.

Gen	SNP	Primers	PCR (T °C)	Enzymes ^a	References
DBP	rs4588	5'GAT CTC GAA GAG GCA TGT TTC 3'	58.8	Styl	
	rs4071	5'GTT GCC TGT GTT CAC AGA CTC 3'		HaeIII	
CYP2R1	rs10741657	5'-GGGAAGAGCAATGACATGGA-3'	61	MnI	
		5'-GCCCTGGAAGACTCATTG-3'			
	rs12794714	5'-GCCATAAGTCCAACCAGGAA-3'	63	FokI	
		5'-GGAAGCTTTGGAGAGCTGAA-3'			
CYP24	rs927650	5'-TGGTTGCATAACACA AACCTA-3'	59	BglII	
		5'-CTGAAAGCCAGTAACAATGGT-3'			
VDR	rs7975232	5'-AGTAAGAGTCTGGCAAAGATAGC-3'	58	ApaI	
	rs731236	5'-AAACACTTCGAGCACAAGG-3'		TaqI	
	rs1544410	5'-GGCAACCTGAAGGGAGACGTA-3'	60	BsmI	
		5'-CTCTTTGGACCTCATCCAGAC-3'			
	rs10735810	5'-AGCTGGCCCTGGCACTGACTCTGTCT-3'	60	FokI	
		5'-ATGGAACACCTTGCTTCTTCCCTC-3'			
Real-Time-PCR ^b					
CYP24	rs2248137	C_1915659			
	rs2296241	C_1915656_30			
CYP27B1	rs10877012	forward: GGGAGTAAGGAGCAGAGAGGTAA reverse: AACAGAGAGAGGGCTGTCT FAM-labelled probe for C allele, TGTGGGAGATTCTTTTA VIC-labelled probe for A allele, CTGTGGGAGATTATTTTA			

The digestion products were separated on 3% agarose gel visualized by ethidiumbromide staining.

^a All enzymes used for the RFLP analyses were purchased from New England Biolabs, Inc., Beverly, MA, USA and used according manufacturer's instructions.

^b Real-Time PCR was analyzed using TaqMan assays in an ABI 7300 PCR (picture not shown) system under the conditions recommended by the manufacturer (Applied Biosystems).

polymorphism within the CYP24 gene showed a higher serum level of the 1,25(OH)₂D₃ in comparison to the patients carrying the genotypes CC or CG ($p < 0.05$). There were no differences in the distribution of the other polymorphisms within the CYP24 or CYP27B1 genes and the serum levels of 1,25(OH)₂D₃.

When vitamin D levels were stratified by the time of year that blood samples were taken, there were no seasonal differences between patients and controls. In addition, there were no correlations between 25(OH)D levels and CD4/CD8 for chemical or clinical characteristics.

4. Discussion

The results of this study showed that patients with ACS had significantly lower vitamin D levels in the first few hours after diagnosis compared with a healthy control group. In addition, SNPs in the genes encoding for CYP24 whose mutation have been associated with infantile hypercalcemia⁴¹ and CYP27B1 were also associated with ACS risk. These polymorphisms might represent a new cardiac risk factor. With our findings it could be postulated that the allele T is associated with an increased risk for ACS development, while on the other hand the genotype GG within the CYP24 rs2296241 SNP occurred less frequently in ACS patients vs controls (7% vs 18%). With genotype AG found more frequently in ACS patients (79% vs 57%, $p < 0.01$) and the homozygous GG less frequently detected, variant AG was also more frequent in ACS patient. Presumably, the allele G might have a protective effect regarding ACS development, while the allele A may represent increased risk.

Although it is not possible to influence or alter genetic polymorphisms, it may be that vitamin D supplementation could overcome the resulting negative effects. The findings do fit well with what is currently known about the pathophysiology of ACS. Vitamin D deficiency might lead to increased coronary calcification and to plaque rupture. Therefore vascular repair by pro-angiogenic cells after ACS may be attenuated in the presence of vitamin D deficiency.

The finding of an association between CYP24 gene polymorphisms and ACS in this study is in agreement with the results of a previous trial showing that CYP24A1 SNPs were associated with the quantity of coronary artery calcification in three independent populations.⁴²

However, the current results contradict those of a large case-control study that failed to find any significant associations between gene polymorphisms within the vitamin D cascade (vitamin D binding protein, CYP2R1, CYP27B1 and CYP24A1) and cardiovascular risk.¹⁶

Epidemiological evidence exists to support a role for vitamin D metabolites in the development of coronary artery calcification, and the risk of coronary artery disease and ACS. Their results suggested a possible role for vitamin D in the development vascular calcification.²³ These data suggest that the increased cardiovascular risk associated with vitamin D deficiency might be, at least partly, due to accelerated development of atherosclerosis. Watson et al. measured serum 1,25(OH)₂D₃ in asymptomatic individuals at high risk of coronary heart disease and in those with familial hypercholesterolaemia, but found inverse correlation⁴³ between serum 1,25(OH)₂D₃ levels and coronary artery calcification in both groups of patients studied.⁴³ Serum levels of 1,25(OH)₂D₃ were also independently and inversely associated with coronary calcium mass in asymptomatic subjects with risk factors for coronary heart disease.²⁴ In another study, low 25(OH)D levels were associated with subsequent development of coronary artery calcification.⁴⁴ On the other hand, a number of other studies showed no association between serum vitamin D levels and coronary calcification.^{11,38,45–50} Such discrepancies in study results with the majority reporting non-beneficial effects mean that the precise role of vitamin D in cardiovascular disease is not yet clear. Nevertheless study design, limit for vitamin D deficiency, age, ethnicity, seasonality among other factors, which may have prevented beneficial vitamin D effects, should be discussed.^{28,51}

Vitamin D deficiency is often seen in clinical practice, but it's implications remain to be determined. A number of studies investigating vitamin D supplementation for the primary prevention of cardiovascular disease have had negative outcomes.^{14,52}

Important limitations of the current study include its retrospective design and the small number of subjects. However, the results do highlight the important role of vitamin D serum levels and may be also of the vitamin D polymorphisms, at least in the first hours after the diagnosis of ACS. Suggestions for future research include a prospective study comparing vitamin D supplementation and placebo for the primary prevention of cardiovascular disease in individuals who have the

Fig. 1. Distribution of the 25(OH)D levels between patients with ACS and healthy controls.

Fig. 1A shows 25 OH vitamin D levels in ng/ml for the 71 patients with ACS and healthy controls (13.2 vs 21.3 ng/mL, $p < 0.05$). Fig. 1B shows the correlation of 1,25 OH vitamin D levels in pg/ μ l and 25 OH vitamin D levels in ng/ml to demonstrate equilibrium in vitamin D metabolism in the study population.

gene polymorphisms identified in this study as being associated with ACS, and secondary prevention trials examining the effects of vitamin D after MI.

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Declaration of competing interest

All authors state that there is no conflict of interest in regard to this study.

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