ORIGINAL ARTICLE

Clinical aspects of vitamin D-binding protein gene polymorphisms in hemodialysis patients

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KEY WORDS

hemodialysis, phosphorus, sex, vitamin D-binding protein gene, 25(OH)D

ABSTRACT

INTRODUCTION There are scarce data on the associations between vitamin D-binding protein gene (*GC*) polymorphisms and manifestations of chronic kidney disease.

OBJECTIVES We evaluated the frequency distribution of *GC* polymorphic variants in hemodialysis (HD) patients and healthy subjects as well as the differences in the prevalence of coronary artery disease (CAD) and myocardial infarction (MI) and selected clinical and laboratory indices of secondary hyperparathyroidism in HD patients (women and men) with different *GC* polymorphic variants.

PATIENTS AND METHODS HD patients (n = 1056; 625 men) and healthy controls (n = 313; 150 men) were enrolled into the study. The tested *GC* polymorphisms included rs2298849, rs7041, and rs1155563. We analyzed clinical data (prevalence of CAD and MI; treatment with parathyroidectomy or cinacalcet) and laboratory results (serum calcium, phosphorus, alkaline phosphatase, parathyroid hormone, and 25-hydroxy vitamin D [25(OH)D] in relation to the gene polymorphisms.

RESULTS There were no differences between the study groups themselves and between the study groups and controls in terms of the frequency distribution of *GC* polymorphisms ($P_{trend} < 0.05$). Lower plasma 25(OH)D levels were shown in subjects with the rs7041 TT genotype compared with those with the GG genotype (12.7, 5.7–20.9 ng/ml vs. 15.9, 8.0–50.0 ng/ml, $P = 0.02$). Women with the rs7041 TT genotype compared with those with the GG genotype showed higher serum phosphorus levels (5.58, 3.40–8.97 mg/dl vs. 5.03, 1.75–9.33 mg/dl, $P = 0.007$).

CONCLUSIONS HD patients do not differ in the distribution of *GC* polymorphisms rs2298849, rs7041, and rs1155563 from healthy subjects. In HD patients, the *GC* polymorphism is associated with plasma 25(OH)D levels. Sex-related factors may be important in the expression of associations between *GC* polymorphic variants and mineral disorders.

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INTRODUCTION 25-hydroxy-vitamin D [25(OH)D] is a widely accepted biomarker of the vitamin D status, also in end-stage renal disease (ESRD).[1] Patients with ESRD, including those on hemodialysis (HD), usually have low plasma vitamin D levels.[2,3] Vitamin D deficiency is epidemiologically linked to life-threatening diseases such as myocardial infarction (MI),[4-6] cerebral stroke,[5] and bone fractures,[7] the prevalence of which is related to sex.[4,8-10] In dialysis patients, the above conditions are more frequent than in the general population. Male sex was shown to be associated with a higher frequency of MI,[11] while mineral bone disorders, including secondary hyperparathyroidism (HPT), were reported as more severe in uremic women than in men.[12-14] Sex-specific effects of vitamin D were also demonstrated in mice.[15]

Decreased plasma 25(OH)D levels may be caused by insufficient levels of circulating vitamin D-binding protein (DBP), which has a spectrum of biological activities (actin scavenging, fatty acid transport, macrophage activation, and chemotaxis) and transports vitamin D to its receptors in target tissues.[16] Lower baseline plasma

DBP levels have been recently related to mortality in HD patients.[17]

DBP is encoded by a group-specific component gene (*GC*) located on chromosome 4q13.3. In sparse data on the *GC* polymorphism in dialysis patients, median serum DBP concentrations were the lowest in HD patients with both rs7041 TT and rs4588 AA genotypes,[18] and the *GC* polymorphism was not associated with response to hepatitis B vaccination[19] or end-stage type 2 diabetic nephropathy.[20]

Gene expression may be mediated by sex. Previous papers have reported a sex-specific association of vitamin D receptor (*VDR*) polymorphism combinations with type 1 diabetes[21] and growth during the first 2 years of life[22] in nonuremic subjects, but sex-dependent associations of *GC* and features of ESRD have not been investigated.

We evaluated the frequency distribution of *GC* polymorphisms in HD patients and healthy subjects and assessed differences in the prevalence of coronary artery disease (CAD), MI, and selected mineral metabolism disturbances of secondary HPT in HD patients with different *GC* polymorphic variants. To our knowledge, this is the first such analysis. Additionally, HD women and men were analyzed separately to explore possible sex-associated phenotype differences in relation to *GC* genotypes. Our findings may have a predictive value for establishing a genetic-related risk of developing ESRD-associated comorbidities and may help explain the differences in clinical and laboratory parameters between men and women on HD.

PATIENTS AND METHODS

Patients and controls A total of 1056 patients on HD (625 men) and 313 healthy controls (150 men) were enrolled into the study. All individuals were Caucasians and lived in the Wielkopolska region of Poland. Healthy individuals (unrelated blood donors and healthy volunteers) served as controls for the frequency distribution of *GC* polymorphic variants.

In HD patients, therapeutic efforts aimed to reach normal serum levels of calcium and phosphorus and to maintain serum intact parathyroid hormone (PTH) levels at a range of approximately 2 to 9 times the upper normal limit for the assay in accordance with the Kidney Disease: Improving Global Outcomes (KDIGO) Work Group clinical practice guidelines.[1] Patients with serum PTH levels exceeding 500 pg/ml received treatment with cinacalcet hydrochloride.[23] The number of patients using cinacalcet was limited by lack of reimbursement from the National Health Fund (Narodowy Fundusz Zdrowia). In HD patients with severe secondary HPT who failed to respond to pharmacological treatment (PTH levels exceeding 1000 pg/ml in repeated evaluations, suspicion of parathyroid gland adenoma on 99mTc-MIBI scintigraphy), parathyroidectomy (PTX) was performed if possible (no clinical contraindications, written informed consent obtained).

Single nucleotide polymorphism selection and genotyping Genomic DNA for a genotype analysis was isolated from peripheral blood lymphocytes by a salt-out extraction procedure. Single nucleotide polymorphisms (SNPs) in the *GC* gene were identified from the HapMap Genome Browser (http://hapmap. ncbi.nlm.nih.gov/), the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/), and related literature. The final set of 3 SNPs was selected based on a minor allele frequency over 15% in the Caucasian population and *GC* gene-linkage disequilibrium (LD) patterns. The loci selected for this study finally included *GC* rs2298849, rs7041, and rs1155563. Their characteristics are presented in *Supplementary material online, Table S1*. The LD pattern and structure of haplotype blocks across the *GC* gene were determined using genotype data from the HapMap database and the Haploview 4.0 software package (http://www.broad.mit.edu/mpg/haploview/). The plot of the pairwise LD between SNPs (r^2 and D' values) in the *GC* gene is presented in *Supplementary material online, Figure S1*.

The genotyping of the rs7041 polymorphism was conducted by polymerase chain reaction (PCR), followed by digestion of the amplified products with the HaeIII (GG/CC) restriction enzyme (PCR-RFLP) and 2% agarose gel electrophoresis. The rs7041 T allele remained uncut (493bp), whereas the rs7041 G allele was cleaved by HaeIII into 414bp and 79bp fragments. The genotyping of rs1155563 and rs2298849 nucleotide variants was conducted by high-resolution melting curve analysis (HRM) on the Bio-Rad CFX96 Real Time PCR system (Bio-Rad, Hercules, California, United States). DNA fragments amplified with the use of specific primers were subjected to HRM with 0.1°C increments in the temperature ranging from 71°C to 83°C. Primer sequences and conditions for PCR-RFLP and HRM analyses are presented in *Supplementary material online, Table S2*. For quality control, the genotyping analysis was blinded to the subject's case-control status. In addition, approximately 10% of the randomly chosen samples were regenotyped.

Genotyping was performed in all study subjects. Samples with ambiguous results were excluded from further statistical analyses.

Clinical and laboratory data Clinical data, obtained from 955 HD patients (565 men), included the prevalence of CAD and MI, frequency of PTX, and frequency of treatment with cinacalcet. CAD was diagnosed based on a medical history, electrocardiograms, exercise stress test, and, in some cases, on coronary angiography or computed tomography. MI was diagnosed based on a medical history and evidence showing characteristic electrocardiographic abnormalities and elevated levels of cardiac markers of cardiomyocyte damage.

Laboratory parameters included serum concentrations of total calcium, phosphorus, and PTH as well as serum activities of total alkaline

phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyltransferase (GGT). They were obtained from 827 HD patients (477 men). Each laboratory parameter was a mean value of 2 to 4 results obtained before the study enrollment depending on HD vintage. In patients who underwent PTX or were treated with cinacalcet, laboratory data obtained before those treatments were included in the analysis. It was not possible to obtain clinical and laboratory data from all patients in some private dialysis facilities because of their intrinsic regulations.

Additionally, plasma 25(OH)D levels were determined in 213 HD patients (120 men) blindly selected from the group of 827 patients who had all other laboratory data available. Blood samples for 25(OH)D were taken in the winter to avoid differences in sunlight exposure between patients who used to sunbathe and those who did not. Plasma 25(OH)D concentrations were measured in HD patients who had not been treated with vitamin D or had stopped such a treatment at least 3 weeks earlier.

Sex distribution was not significantly different between all HD patients (n = 1056), those who had all clinical data available (n = 955), those who had all laboratory data available (n = 827), and those tested for plasma 25(OH)D levels (n = 213).

Clinical and laboratory data were compared in HD patients with different polymorphic variants of the analyzed genotypes.

Laboratory methods To examine plasma 25(OH)D levels, a chemiluminescent microparticle immunoassay was used according to the manufacturer's instructions (Abbot Diagnostic ARCHITECT 25-OH VITAMIN D CMIA®, Mannheim, Germany). Other laboratory parameters were measured using standard laboratory assays.

Statistical methods The results are presented as percentage for categorical variables, mean with 1 standard deviation for normally distributed continuous variables, or median (range) for nonnormally distributed continuous variables as tested by the Shapiro–Wilk test. A *P* value of less than 0.05 was considered significant.

The Hardy–Weinberg equilibrium (HWE) was tested to compare the observed genotype frequencies with the expected ones using the χ^2 test. Distributions of the tested polymorphisms were consistent with the HWE with the exception of *GC* rs1155563 in the entire HD group and in men. Repeated genotyping in all subjects with *GC* rs1155563 confirmed this lack of consistency (the rate of concordance between both analyses was 99.7%).

The Fisher exact probability test or the χ^2 test was used to evaluate the differences in genotype and allele prevalence between the groups. The odds ratios (ORs) with *P* values and 95% confidence intervals (CIs) were calculated. Polymorphisms were tested for associations using the χ^2 test for trend (P_{trend}). A power analysis was performed using the Fisher exact test.

The whole group of HD patients as well as HD women and HD men separately were divided according to their *GC* polymorphic variants for screening for phenotype–genotype associations. ORs with *P* values and 95% CIs were calculated using the contingency table for each tested phenotype separately, if it was a dichotomous variable. All probabilities were 2-tailed. For continuous variables, the Mann–Whitney test, *t* test, or Cochran–Cox test was used, as appropriate. Three models (dominant, recessive, and additive) of inheritance were applied for each *GC* polymorphism. The *P* values with the Bonferroni correction for multiple testing were considered significant if a *P* value was lower than 0.017 (1 SNP, 3 models, 1 phenotype). Associations shown to be significant were tested together in selected groups of HD patients using the Bonferroni-corrected *P* values. In this case, a *P* value of 0.05 was divided by the product of the number of polymorphisms, number of basic models, and number of dependent variables of interest as described by Wypasek et al.[24] Associations that remained significant were analyzed using a logistic regression to show the magnitude of the genotype effect in relation to the 50th percentile of continuous variables. Covariates used for an adjustment were age and sex, where appropriate.

Multiple regression analysis was performed to determine whether genotypes shown as associated with specific phenotypes were also independent predictors of those phenotypes among other variables. The multivariable models included clinical characteristics of HD patients, laboratory parameters related to vitamin D and secondary HPT, and a *GC* polymorphic variant tested for associations. The number of variables included into the model depended on the size of the sample tested.

Statistical analysis was performed using GraphPad InStat 3.10, 32 bit for Windows, created July 9, 2009 (GraphPad Software, Inc., San Diego, California, United States), CytelStudio version 10.0, created January 16, 2013 (CytelStudio Software Corporation, Cambridge, Massachusetts, United States), and Statistica version 10, 2011 (Stat Soft, Inc., Tulsa, Oklahoma, United States).

A haplotype-based association analysis using a sliding window approach was performed using the Haploview 4.2 software. Statistical significance was assessed using the 1000-fold permutation test.

Ethical approval The research design was approved by the Institutional Review Board of the Poznan University of Medical Sciences, Poland. Informed consent was obtained from all study participants.

RESULTS The main clinical and laboratory data of HD patients are presented in **TABLE 1**. Men showed a significantly higher prevalence of CAD

TABLE 1 Characteristics of women and men treated with intermittent hemodialysis

Parameter		Women, n = 431	Men, n = 625	*P* value
demographic data		n = 431	n = 625	
age, y		63.4 ±14.8	61.3 ±14.8	0.6[a]
RRT duration, y		2.96 (0.05–26.1)	2.46 (0.07–28.0)	0.3[a]
main causes of end-stage renal disease	diabetic nephropathy	126 (29)	176 (28)	0.7[b]
	hypertensive nephropathy	72 (17)	119 (19)	0.4[b]
	chronic glomerulonephritis	56 (13)	96 (15)	0.3[b]
	chronic interstitial nephritis	46 (11)	63 (10)	0.8[b]
clinical data		n = 390	n = 565	
coronary artery disease		128 (33)	229 (41)	0.02[b]
myocardial infarction		63 (16)	139 (25)	0.002[b]
PTX		16 (4)	12 (2)	0.08[b]
cinacalcet treatment		68 (17)	85 (15)	0.3[b]
PTX and/or cinacalcet treatment		84 (22)	97 (17)	0.09[b]
laboratory data		n = 350	n = 477	
25(OH)D, ng/ml[c]		11.8 (4.51–50.0)	15.5 (5.10–30.1)	0.000003[a]
total calcium, mg/dl		8.94 ±0.77	8.84 ±0.76	0.008[a]
phosphorus, mg/dl		5.03 (1.75–11.27)	5.08 (1.95–12.0)	0.9[a]
PTH, ng/l		385 (13.7–3,741)	376 (7.3–3,757)	0.9[a]
total ALP, U/l		99.1 (25.8–1.684)	92.3 (38.3–977)	0.003[a]
total ALP (U/l) of patients with ALT, ASP, or GGT ≤45 U/l[d]		98.3 (25.8–1.299)	86.5 (40.5–977)	0.0004[a]

Data are presented as mean ± standard deviation, median (interquartile range), or number (percentage).

Statistical tests: **a** Mann–Whitney, **b** χ^2, **c** n = 93 for women, n = 120 for men, **d** n = 237 for women, n = 299 for men

Conversion factors to SI units are as follows: for 25(OH)D, 1 ng/ml = 2.496 nmol/l; for calcium, 1 mg/dl = 0.25 mmol/l; and for phosphorus, 1 mg/dl = 0.323 mmol/l

Abbreviations: ALP, total alkaline phosphatase; AST, alanine aminotransferase; GGT, γ-glutamyltransferase; HD, hemodialysis; PTH, parathyroid hormone; PTX, parathyroidectomy; RRT, renal replacement therapy

and MI, while women had lower serum 25(OH)D concentrations and higher serum calcium levels and ALP activity than men.

The analysis of HapMap CEU data showed that common (minor allele frequency, >0.2) *GC* SNPs resided in 2 distinct LD blocks (*Supplementary material online, Figure S1*). Within the LD blocks, the D' and r^2 values ranged from 0.73 to 1.00 and 0.42 to 1.00, respectively. The rs7041 and rs1155563 variants were in moderate pairwise LD with other SNPs within the first LD block (average r^2 = 0.77 and 0.61, respectively) and were good proxies (r^2 ≥0.8) for 4 of 12 common variants. The rs2298849 variant, located within the second LD block, was in perfect LD (r^2 = 1) with the intronic variant, rs1352845 (*Supplementary material online, Figure S1*).

Compared with healthy controls, HD patients did not differ in the frequency distribution of the tested *GC* polymorphic variants (**TABLE 2**). In addition, no differences were observed between the groups in relation to sex (*Supplementary material online, Tables S3–5*).

The haplotype analysis of *GC* polymorphisms did not show any differences in haplotype frequencies between any of the study groups (*Supplementary material online, Table S6*).

The results of screening for phenotype–genotype associations are presented in *Supplementary material online, Tables S7–S15*. In all studied HD groups, no associations were revealed between *GC* polymorphic variants and clinical parameters (prevalence of CAD and MI; treatment with PTX or cinacalcet) and the majority of laboratory data (PTH and total calcium concentrations, total ALP activity). The only results suggesting an association with *GC* polymorphic variants were those for plasma 25(OH)D concentrations in the entire HD group and serum phosphorus levels in HD women.

HD patients showing the *GC* rs7041 TT genotype revealed significantly lower plasma 25(OH)D concentrations compared with those showing the *GC* rs7041 GG genotype (**TABLE 3**). HD individuals with the *GC* rs7041 TT genotype had an approximately 2-fold higher risk for plasma 25(OH)D levels below the 50the percentile (13.9 ng/ml) than HD subjects showing the *GC* rs7041 GG genotype (OR, 0.51; 95% CI, 0.29–0.88; sex- and age-adjusted *P*, 0.014). *GC* rs7041 GG or TT genotypes were used in the multiple regression analysis to investigate their predictive value among other variables in respect to 25(OH)D concentrations. The first model included the basic

TABLE 2 Comparison of the distribution of vitamin D-binding protein genotypes (*GC*) in hemodialysis patients and healthy controls

GC	HD patients (frequency)	Healthy controls (frequency)	Odds ratio (95% CI)	2-tailed *P*	P_{trend}	$P_{genotyping}$	Power (%)
rs2298849	n = 1050	n = 309					
TT	669 (0.64)	185 (0.60)	reference	–	0.5	0.2	
CT	327 (0.31)	112 (0.36)	0.807 (0.612–1.068)	0.1			33.3
CC	54 (0.05)	12 (0.04)	1.244 (0.641–2.610)	0.6			7.3
CT+CC	381 (0.36)	124 (0.40)	0.850 (0.650–1.113)	0.2			22.1
MAF	435 (0.21)	136 (0.22)	0.926 (0.742–1.160)	0.5			10.4
P for HWE	0.243	0.617					
rs7041	n = 1001	n = 292					
GG	341 (0.35)	97 (0.33)	reference	–	0.8	0.6	
GT	485 (0.48)	150 (0.52)	0.920 (0.680–1.241)	0.6			8.0
TT	175 (0.17)	45 (0.15)	1.106 (0.732–1.689)	0.7			7.0
GT+TT	660 (0.65)	195 (0.67)	0.963 (0.722–1.279)	0.8			5.3
MAF	835 (0.42)	240 (0.41)	1.026 (0.847–1.243)	0.8			5.4
P for HWE	0.994	0.580					
rs1155563	n = 1054	n = 311					
TT	513 (0.49)	156 (0.50)	reference	–	0.2	0.2	
CT	415 (0.39)	129 (0.42)	0.978 (0.743–1.290)	0.9			5.2
CC	126 (0.12)	26 (0.08)	1.474 (0.920–2.431)	0.1			36.7
CT+CC	541 (0.51)	155 (0.50)	1.061 (0.817–1.378)	0.7			6.8
MAF	667 (0.32)	181 (0.29)	1.128 (0.924–1.380)	0.2			12.7
P for HWE	0.014	0.996					

Abbreviations: CI, confidence interval; HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency

TABLE 3 Serum 25(OH)D concentrations in all hemodialysis patients in relation to *GC* rs7041 polymorphic variants

Parameter	*GC* rs7041			Model of inheritance	*P* value
	GG, n = 76	GT, n = 96	TT, n = 37		
25(OH)D, ng/ml	15.9 (8.0–50.0)	13.9 (4.5–28.5)	12.7 (5.7–20.9)	GG vs GT + TT:	0.04[a,b]
				GG + GT vs TT:	0.05[a]
				GG vs TT:	0.016[a,c]

Data are presented as median (range).

a Mann–Whitney test, **b** nonsignificant after the Bonferroni correction ($P > 0.017$), **c** significant after the Bonferroni correction ($P < 0.017$)

For conversion factors to SI units, see **TABLE 1**

characteristics of HD patients: age, renal replacement therapy (RRT) vintage, prevalence of the main causes of renal failure (diabetic nephropathy, hypertensive nephropathy, chronic glomerulonephritis, and chronic tubulointerstitial nephritis); CAD as associated with genetic variants related to the vitamin D pathway[25]; laboratory parameters related to vitamin D and secondary HPT: calcium, phosphorus, PTH, total ALP as well as and *GC* rs7041 GG genotype (*P* value for the model, <0.0001). Significant positive predictors of 25(OH)D concentrations among RRT patients were serum calcium levels (β = 0.174; *P* = 0.002) and the *GC* rs7041 GG genotype (β = 0.247; *P* = 0.0005), whereas older age (β = –0.225; *P* = 0.004) and RRT vintage were negative predictors (β = –0.263; *P* = 0.001). In the second model, the *GC* rs7041 GG genotype was replaced by the *GC* rs7041 TT polymorphic variant (*P* value for the model, 0.0002). The *GC* rs7041 TT genotype was a significant negative predictor of 25(OH)D (β = –0.150; *P* = 0.04); other variables yielded similar significance as in the first model. However, lower plasma 25(OH)D concentrations in HD women compared with men occurred independently of the rs7041 polymorphic variant (*P* = 0.008 for the GG group; *P* = 0.002 for the GT group; *P* = 0.009 for the TT group; Mann–Whitney test).

When HD patients were divided according to sex, the only parameter possibly associated with the *GC* polymorphism was that of serum phosphorus levels in relation to *GC* rs7041 polymorphic variants in HD women. *GC* rs7041 TT women compared with GG ones showed higher serum

TABLE 4 Plasma 25(OH)D concentration and serum phosphorus levels in hemodialysis women in relation to *GC* rs7041 polymorphic variants

Parameter	*GC* rs7041			Odds ratio (95% CI)	*P* value
	GG	GT	TT		
25(OH)D, ng/ml	12.6 (8.0–50.0)	11.8 (4.5–20.3)	11.3 (5.8–15.7)	GG vs GT + TT:	0.2[a]
	n = 32	n = 42	n = 17	GG + GT vs TT:	0.06[a]
				GG vs TT:	0.04[a,b]
phosphorus, mg/dl	5.0 (1.8–9.3)	4.8 (2.2–11.3)	5.6 (3.4–9.0)	GG vs GT + TT:	0.2[a]
	n = 113	n = 165	n = 49	GG + GT vs TT:	0.008[a,b]
				GG vs TT:	0.007[a,c]

Data are presented as median (range).

a Mann–Whitney test, **b** nonsignificant after the Bonferroni correction ($P > 0.008$), **c** significant after the Bonferroni correction ($P < 0.008$)

For conversion factors to SI units, see **TABLE 1**

Abbreviations: see **TABLE 2**

phosphorus levels (*Supplementary material online, Table S8*). Women with the *GC* rs7041 TT genotype showed a 1.5-fold higher risk for serum phosphorus concentrations over the 50th percentile (5.07 mg/dl) than HD women with the *GC* rs7041 GG genotype (OR, 1.55; 95% CI, 1.05–2.31; age-adjusted $P = 0.028$). A significant association previously shown between the *GC* rs7041 polymorphism and plasma 25(OH)D concentrations in all HD patients (**TABLE 3**) was not observed either in men or women (*Supplementary material online, Tables S8 and S11*), probably owing to a lower power after dividing the whole tested group according to sex. Assuming that this significance could be observed in larger samples of both sexes, 2 phenotypes together [25(OH)D and phosphorus] were also tested in women for association with *GC* rs7041. Addition of another phenotype did not abolish the significance of an association between *GC* rs7041 and serum phosphorus levels, although the Bonferroni-corrected *P* value required to confirm significance had to be lower (**TABLE 4**).

Diabetic nephropathy (β, 0.208; $P = 0.03$), serum PTH levels (β, 0.229; $P = 0.02$), and the *GC* rs7041 TT genotype (β, 0.197; $P = 0.04$) were positive predictors, while age was a negative predictor (β, –0.259; $P = 0.01$) of serum phosphorus levels in HD women. Other predictors used in this model ($P < 0.0005$) included RRT vintage, hypertensive nephropathy, 25(OH)D, and calcium. The number of variables used in this model was smaller because the group was divided into subgroups according to sex, which reduced the number of subjects available for the regression analysis.

An association was also suspected between 25(OH)D and rs1155563, but the level of significance was not achieved (*Supplementary Tables 12 and 15*).

DISCUSSION Dialysis patients are at a higher risk of CAD and MI compared with healthy individuals.[26] Associations of genetic variants related to the vitamin D pathway (rs7968585 *VDR*, rs2239179 *VDR*, rs1801222 *CUBN*, rs12766939 *CUBN*, and rs703842 *CYP27B1*) and MI were identified in individuals with a mean estimated glomerular filtration rate exceeding 70 ml/min/1.73 m^2.[25] In our study on patients with severely impaired renal function diagnosed as ESRD, there was no association of the examined *GC* polymorphisms and CAD/MI occurrence, independently of whether all HD patients were analyzed or men and women separately.

Several studies have shown that the *GC* polymorphism may contribute to the variation of serum 25(OH)D levels.[18,27-34] In a genome-wide association study, 3 SNPs in the *GC* (rs2282679, rs7041, and rs1155563) were identified as associated with 25(OH)D concentrations in the European population. The strongest association was observed for rs2282679, which is in moderate LD with rs7041.[32] According to genotype data from HapMap CEU samples, the r^2 and D' values for this pair of SNPs were 0.61 and 1.00, respectively.[32] In our study, lower 25(OH)D levels were shown in HD patients with the rs7041 TT genotype compared with carriers of the rs7041 GG polymorphic variant, and *GC* rs7041 was predictive for plasma 25(OH)D levels. Therefore, our results are in line with the earlier findings indicating that individuals who have 2 T alleles of the rs7041 missense variant are at risk for vitamin D deficiency. Additionally, we showed that the association of rs7041 with 25(OH)D was also evident in uremic milieu. A substitution of aspartic acid to glutamic acid at amino acid position 432 (Asp432Glu) of DBP attributed to the functional properties of the *GC* rs7041 variant located in exon 11 seems to be an important contributor to decreased plasma 25(OH)D levels, possible by inducing a lower affinity of 25(OH)D to DBP. HD women showed lower plasma 25(OH)D concentrations than men. However, the association of the rs7041 TT genotype with lower plasma 25(OH)D levels does not explain lower 25(OH)D levels

in HD women compared with men because women showed lower 25(OH)D levels at all 3 rs7041 genotypes.

Similarly to *GC* rs7041, also *GC* rs1155563 is known to be associated with plasma 25(OH)D levels.[32] In HD subjects, specifically in men, this association had borderline significance. However, in all HD patients, and separately in men, the distribution of polymorphic variants of *GC* rs1155563 was not consistent with the HWE. In our previous study, the nonconsistency of *GC* rs1155563 polymorphic variants with the HWE was shown in nonresponders to hepatitis B vaccination, although genotyping was also repeated.[19] It is not clear why HD subjects show deviations in this polymorphism.

Mineral bone disorders, including secondary HPT, are reported to be more severe in dialysis women than in men.[12,13] Serum intact PTH concentrations do not always allow to assess the presence or severity of secondary HPT.[1] In our study, serum PTH levels were similar in women to those in men even though PTH levels before PTX or cinacalcet treatment were included in the analysis. On the other hand, PTX and cinacalcet were used for secondary HPT treatment more often in women compared with men (however, statistical significance was borderline). Repeated measurements of total ALP activity are recommended for HD patients without clinically evident liver disorders to determine the bone turnover status as indicative of secondary HPT severity.[1] Considering the possibility of an increasing contribution of the liver-derived isoenzyme of ALP to its total activity in the case of increased activities of other liver enzymes, we additionally analyzed the serum activity of total ALP with the exclusion of patients with ALT, AST, and GGT activities exceeding 45 U/l. This analysis confirmed a higher total ALP activity in women than in men, which suggests a higher bone turnover related to secondary HPT in the female group. In patients undergoing PTX due to secondary HPT, women had lower preoperative bone mineral density than men, although no differences in the preoperative calcium–phosphorus product, ALP, or PTH were observed.[12] Significantly higher calcium concentrations in HD women could reduce differences in serum PTH levels compared with HD men, although serum ALP activities indicated more severe secondary HPT in women. We did not observe any associations between the *GC* polymorphisms and PTH levels or ALP activity. However, HD women with the rs7041 TT genotype showed higher serum phosphorus levels compared with those positive for the rs7041 GG genotype. No similar observation was made in HD men. It indicates that minor allele T rs7041 homozygosity in HD women is associated with abnormalities promoting secondary HPT, namely, hyperphosphatemia and low plasma levels of 25(OH)D. Hyperphosphatemia appears to be particularly important because high phosphorus concentrations can directly stimulate PTH synthesis.[35] In HD patients, hyperphosphatemia contributes directly to an increase in PTH levels despite normal or high serum calcium concentrations.[36]

The question remains what might be the possible link between *GC* rs7041 polymorphic variants and serum phosphorus concentrations only in women. In ESRD patients, vitamin D deficiency is frequently seen concomitantly with hyperphosphatemia. Although the causes of hyperphosphatemia in ESRD are multifactorial, it cannot be excluded that both low vitamin D levels and skeletal resistance to PTH action shown in uremic milieu (partially due to low vitamin D)[37] may contribute to increased phosphorus levels. The development of PTH resistance is related to female sex.[38] However, there is no clear explanation how the phenotypes related to vitamin D itself or vitamin D pathway genes are mediated by sex. Studies showing sex-specific association of *VDR* polymorphisms with type 1 diabetes[21] or growth during the first 2 years of life[22] did not provide any explanation. Experimental studies in mice suggest that there is synergy between ovarian hormones and vitamin D.[15] We might postulate that higher phosphorus levels in HD women with the *GC* rs7041 TT genotype, but not men, may be related to the effects of ovarian hormones on the expression of this specific *GC* polymorphic variant.

Our study has several limitations. First, the study groups were relatively small, especially when divided into subgroups according to sex. It made it difficult to obtain statistical significance in comparisons that included the data on minor allele homozygosity, and when the Bonferroni correction for multiple comparison was used. On the other hand, the Bonferroni correction[39] has been criticized, and not all authors use it in genetic studies, especially when the established associations are investigated in more detail or in a more uniform cohort.[40,41] The *GC* rs7041 TT genotype has already been associated with lower plasma 25(OH) concentrations,[27-33] but in HD subjects, only in combination with the rs4588 AA genotype.[18] The HD group in our study was heterogeneous with regards to the causes of ESRD. However, the development of ESRD substantially reduces clinical variability between patients related to underlying kidney damage and exposes the signs and symptoms related to uremic toxicity. Our recent study has shown that patients with end-stage type 2 diabetic nephropathy did not differ in the frequency distribution of *GC* polymorphic variants from controls, patients with other causes of ESRD as a whole, as well as subjects with chronic glomerulonephritis, chronic infective tubulointerstitial nephritis, and hypertensive nephritis, showing glomerular filtration category 5 and requiring RRT.[20] However, differences in this respect between other specific causes of ESRD have not been excluded so far. Second, the number of selected polymorphisms in our study was insufficient to fully cover the *GC* gene. The analyzed variants were not good or perfect proxies ($r^2 \geq 0.8$ or 1.00, respectively) for all other

common *GC* SNPs. Therefore, some modest single SNP and/or haplotype associations may have been missed. Finally, 25(OH)D levels were not measured in all subjects for financial constraints.

In summary, HD patients do not differ in the distribution of *GC* polymorphisms rs2298849, rs7041, and rs1155563 from healthy subjects. HD patients show an association between the *GC* rs7041 polymorphism and plasma 25(OH)D levels. Sex-related factors may be important for studying associations between *GC* rs7041 polymorphic variants and serum phosphorus concentrations. The clinical relevance of these findings, especially the association between the *GC* rs7041 polymorphism and sex-related severity of secondary HPT, requires further research.

Supplementary material online Supplementary material online is available with the online version of the article at www.pamw.pl.

Contribution statement AEG conceived the study concept and wrote the manuscript. PJ contributed to the design of the research. AEG and GO were involved in data collection. AM was responsible for genotyping. AS was responsible for statistics. All authors edited and approved the final version of the manuscript.

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Kliniczne aspekty polimorfizmów genu białka wiążącego witaminę D u hemodializowanych chorych

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SŁOWA KLUCZOWE

fosforany, gen wiążący witaminę D, hemodializa, płeć, 25(OH)D

STRESZCZENIE

WPROWADZENIE Istnieją tylko nieliczne badania powiązań polimorfizmów genu białka wiążącego witaminę D (*GC*) z objawami przewlekłej choroby nerek.

CELE Oceniono częstość dystrybucji wariantów polimorficznych *GC* u chorych hemodializowanych (HD) i osób zdrowych, a także różnice w zapadalności na chorobę wieńcową (*coronary artery disease* – CAD) i zawał serca (*myocardial infarction* – MI) oraz wybranych cech kliniczno-laboratoryjnych wtórnej nadczynności przytarczyc u chorych HD (kobiet i mężczyzn) wykazujących różne warianty polimorficzne *GC*.

PACJENCI I METODY Do badania włączono chorych HD (n = 1056, 625 mężczyzn) i osoby zdrowe (n = 313, 150 mężczyzn). Badane polimorfizmy *GC* obejmowały rs2298849, rs7041 i rs1155563. Analizowano dane kliniczne (zapadalność na CAD i MI, częstość paratyroidektomii i leczenia cynakalcetem) oraz wyniki laboratoryjne (stężenie wapnia, fosforu, fosfatazy alkalicznej, parathormonu i 25-hydroksy witaminy D [25(OH)D]) w odniesieniu do badanych polimorfizmów.

WYNIKI Badane grupy nie różniły się pod względem częstości dystrybucji polimorfizmów *GC* ani między sobą, ani w porównaniu z osobami zdrowymi ($p_{trend} < 0,05$). U osób z genotypem rs7041 TT wykazano niższe stężenia 25(OH)D niż u osób, które wykazywały genotyp GG (12,7; 5,7–20,9 ng/ml *vs* 15,9; 8,0–50,0 ng/ml; p = 0,02). Kobiety posiadające genotyp rs7041 TT w porównaniu z kobietami z genotypem GG wykazywały wyższe stężenie fosforanów w surowicy (5,58; 3,40–8,97 mg/dl *vs* 5,03; 1,75–9,33 mg/dl; p = 0,007).

WNIOSKI Pacjenci leczeni hemodializami nie różnią się pod względem dystrybucji polimorfizmów *GC* (rs2298849, rs7041 oraz rs1155563) od osób zdrowych. U chorych HD występuje powiązanie między polimorfizmem *GC* i stężeniem 25(OH)D w osoczu. Czynniki zależne od płci mogą być ważne w ekspresji powiązań między wariantami polimorficznymi *GC* a zaburzeniami mineralnymi.

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Supplementary Material Online

CLINICAL ASPECTS OF VITAMIN D-BINDING PROTEIN GENE POLYMORPHISMS IN HEMODIALYSIS PATIENTS

Supplementary Table 1. Characteristics of the analyzed polymorphisms in the gene encoding vitamin D-binding protein (*GC*)

rs no.	Location	Alleles	SNP function[a]	MAF[b]
rs7041	chr4:72618334	G/T	missense (p.Asp432Glu)	0.42
rs1155563	chr4:72643488	C/T	Intron	0.25
rs2298849	chr4:72648851	C/T	Intron	0.19

a – according to the Single Nucleotide Polymorphism database (dbSNP)

b – calculated from 1000 Genomes project for EUR samples

Abbreviations: MAF – minor allele frequency, SNP – single nucleotide polymorphism

Supplementary Table 2. HRM and RFLP conditions for the identification of the *GC* polymorphisms

					HRM analysis	RFLP analysis	
rs no.	Alleles	Primers for PCR amplification (5' – 3')	Annealing temp. (°C)	PCR product length (bp)	Melting temp. range (°C)	Restriction enzyme	Restriction fragment length (bp)
rs7041	G/T	F: GGAGGTGAGTTTATGGAACAGC	66.3	493		HaeIII	T = 493
		R: GGCATTAAGCTGGTATGAGGTC					G = 414 + 79
rs1155563	C/T	F: GGTTATTCTAAGACTGTGCTCTTGC	63.0	116	71 - 78		
		R: ATGTGTTCTCACTGTTCGACTCC					
rs2298849	C/T	F: TCCACTGGCAAAACACATTAC	60.6	118	73 - 83		
		R: GGGACATCTGCATTTATCCTG					

Abbreviations: HRM – high resolution melt, RFLP – restriction fragment length polymorphism

Supplementary Table 3. Comparison of the distribution of vitamin D-binding protein genotypes (*GC*) in hemodialysis women and men

GC	HD women (frequency)	HD men (frequency)	Odds ratio (95% CI)	2-tailed *P*	P_{trend}	$P_{genotyping}$	Power (%)
rs2298849	n = 429	n = 621					
TT	271 (0.63)	398 (0.64)	reference	–	0.7	0.9	
CT	135 (0.32)	192 (0.31)	1.033 (0.782–1.363)	0.9			5.3
CC	23 (0.05)	31 (0.05)	1.090 (0.593–1.978)	0.9			5.3
CT+CC	158 (0.37)	223 (0.36)	1.041 (0.799–1.354)	0.8			5.7
MAF	181 (0.21)	254 (0.20)	1.040 (0.834–1.295)	0.8			6.2
P for HWE	0.526	0.464					
rs7041	n = 401	n = 600					
GG	134 (0.33)	207 (0.34)	reference	–	0.6	0.3	
GT	205 (0.51)	280 (0.47)	1.131 (0.845–1.516)	0.4			12.7
TT	62 (0.16)	113 (0.19)	0.848 (0.569–1.257)	0.4			12.6
GT+TT	267 (0.67)	393 (0.66)	1.050 (0.797–1.384)	0.8			6.0
MAF	329 (0.41)	506 (0.42)	0.954 (0.792–1.148)	0.6			7.9
P for HWE	0.527	0.572					
rs1155563	n = 430	n = 624					
TT	202 (0.47)	311 (0.50)	reference	–	0.5	0.6	
CT	176 (0.41)	239 (0.38)	1.134 (0.864–1.488)	0.4			14.3
CC	52 (0.12)	74 (0.12)	1.082 (0.712–1.637)	0.8			6.2
CT+CC	228 (0.35)	313 (0.50)	1.122 (0.870–1.445)	0.4			13.9
MAF	280 (0.33)	387 (0.31)	1.074 (0.887–1.299)	0.5			11.3
P for HWE	0.370	0.032					

Abbreviations: CI – confidence interval, HWE – Hardy–Weinberg equilibrium, others – see TABLE 1

Supplementary Table 4. Comparison of the distribution of vitamin D-binding protein genotypes (*GC*) in hemodialysis women and control healthy women

GC	HD women (frequency)	Control women (frequency)	Odds ratio (95% CI)	2-tailed *P*	P_{trend}	$P_{genotyping}$	Power (%)
rs2298849	n = 429	n = 162					
TT	271 (0.63)	87 (0.54)	reference	–	0.1	0.06	
CT	135 (0.32)	68 (0.42)	0.637 (0.429–0.949)	0.03[a]			62.7
CC	23 (0.05)	7 (0.04)	1.055 (0.420–3.013)	1.0			3.5
CT+CC	158 (0.37)	75 (0.46)	0.676 (0.462–0.993)	0.05			54.1
MAF	181 (0.21)	82 (0.25)	0.789 (0.580–1.080)	0.1			33.0
P for HWE	0.526	0.374					
rs7041	n = 401	n = 158					
GG	134 (0.33)	56 (0.35)	reference	–	0.8	0.9	
GT	205 (0.51)	77 (0.49)	1.113 (0.724–1.704)	0.7			7.7
TT	62 (0.16)	25 (0.16)	1.036 (0.574–1.901)	1.0			4.4
GT+TT	267 (0.67)	102 (0.65)	1.094 (0.727–1.636)	0.7			6.8
MAF	329 (0.41)	127 (0.40)	1.035 (0.788–1.363)	0.9			5.3
P for HWE	0.527	0.985					
rs1155563	n = 430	n = 162					
TT	202 (0.47)	76 (0.47)	reference	–	0.5	0.3	
CT	176 (0.41)	73 (0.45)	0.907 (0.610–1.350)	0.7			6.9
CC	52 (0.12)	13 (0.08)	1.505 (0.754–3.184)	0.3			19.6
CT+CC	228 (0.35)	86 (0.53)	0.998 (0.683–1.456)	1.0			4.2
MAF	280 (0.33)	99 (0.31)	1.097 (0.826–1.463)	0.6			9.2
P for HWE	0.370	0.734					

a – nonsignificant after the Bonferroni correction (*P* >0.02)
Abbreviations: see TABLES 1 and 3

Supplementary Table 5. Comparison of the distribution of vitamin D-binding protein genotypes (*GC)* in hemodialysis (HD) men and control healthy men

GC	HD men (frequency)	Control men (frequency)	Odds ratio (95% CI)	2-tailed *P*	P_{trend}	$P_{genotyping}$	Power (%)
rs2298849	n = 621	n = 147					
TT	398 (0.64)	98 (0.67)	reference	–	0.4	0.7	
CT	192 (0.31)	44 (0.30)	1.074 (0.713–1.636)	0.8			5.8
CC	31 (0.05)	5 (0.03)	1.527 (0.568–5.156)	0.5			8.7
CT+CC	223 (0.36)	49 (0.33)	1.121 (0.756–1.677)	0.6			8.0
MAF	254 (0.20)	54 (0.18)	1.143 (0.819–1.615)	0.5			11.1
P for HWE	0.464	1.000					
rs7041	n = 600	n = 134					
GG	207 (0.34)	41 (0.31)	reference	–	1.0	0.2	
GT	280 (0.47)	73 (0.54)	0.760 (0.484–1.181)	0.2			23.2
TT	113 (0.19)	20 (0.15)	1.119 (0.607–2.118)	0.8			5.3
GT+TT	393 (0.66)	93 (0.69)	0.837 (0.544–1.273)	0.4			12.3
MAF	506 (0.42)	113 (0.42)	1.000 (0.759–1.321)	1.0			4.7
P for HWE	0.572	0.400					
rs1155563	n = 624	n = 149					
TT	311 (0.50)	80 (0.54)	reference	–	0.3	0.5	
CT	239 (0.38)	56 (0.37)	1.098 (0.738–1.640)	0.7			6.6
CC	74 (0.12)	13 (0.09)	1.464 (0.757–3.024)	0.3			18.1
CT+CC	313 (0.50)	69 (0.46)	1.167 (0.803–1.698)	0.5			12.5
MAF	387 (0.31)	82 (0.28)	1.184 (0.888–1.589)	0.3			20.5
P for HWE	0.032	0.780					

Abbreviations: see TABLES 1 and 3

Supplementary Table 6. Haplotype analysis of *GC* polymorphisms

HD all vs. CONTROL all

Polymorphisms	Haplotypes	Frequency	Case, control ratios	χ^2	*P* value	P_{corr} value[a]
rs7041_rs1155563	GT	0.537	0.536, 0.542	0.060	0.8	1.0
	TC	0.261	0.267, 0.241	1.736	0.2	0.5
	TT	0.153	0.148, 0.168	1.423	0.2	0.6
	GC	0.049	0.048, 0.050	0.016	0.9	1.0
rs1155563_rs2298849	TT	0.511	0.509, 0.515	0.056	0.8	1.0
	CT	0.279	0.284, 0.265	0.879	0.3	0.7
	TC	0.180	0.175, 0.195	1.246	0.3	0.6
	CC	0.030	0.032, 0.026	0.545	0.5	0.8
rs7041_rs1155563_rs2298849	GTT	0.392	0.395, 0.382	0.315	0.6	1.0
	TCT	0.239	0.244, 0.225	0.879	0.3	0.9
	GTC	0.145	0.141, 0.159	1.304	0.3	0.8
	TTT	0.118	0.113, 0.132	1.579	0.2	0.7
	GCT	0.041	0.042, 0.040	0.026	0.9	1.0
	TTC	0.036	0.035, 0.036	0.013	0.9	1.0
	TCC	0.022	0.023, 0.015	1.448	0.2	0.7

HD women vs. CONTROL women

Polymorphisms	Haplotypes	Frequency	Case, control ratios	χ^2	*P* value	P_{corr} value[a]
rs7041_rs1155563	GT	0.540	0.538, 0.544	0.037	0.8	1.0
	TC	0.265	0.271, 0.252	0.425	0.5	0.9
	TT	0.141	0.137, 0.152	0.402	0.5	0.9
	GC	0.054	0.054, 0.052	0.016	0.9	1.0
rs1155563_rs2298849	TT	0.493	0.500, 0.476	0.540	0.5	0.8
	CT	0.285	0.290, 0.273	0.362	0.5	0.9
	TC	0.188	0.175, 0.219	3.018	0.08	0.2
	CC	0.034	0.035, 0.032	0.046	0.8	1.0
rs7041_rs1155563_rs2298849	GTT	0.384	0.392, 0.362	0.892	0.3	0.9
	TCT	0.246	0.250, 0.237	0.221	0.6	1.0
	GTC	0.155	0.145, 0.182	2.403	0.1	0.4
	TTT	0.106	0.104, 0.111	0.116	0.7	1.0
	GCT	0.043	0.044, 0.039	0.164	0.7	1.0
	TTC	0.036	0.034, 0.041	0.364	0.5	1.0
	TCC	0.019	0.021, 0.015	0.383	0.5	1.0
	GCC	0.011	0.010, 0.013	0.234	0.6	1.0

HD men vs. CONTROL women

Polymorphisms	Haplotypes	Frequency	Case, control ratios	χ^2	*P* value	P_{corr} value[a]
rs7041_rs1155563	GT	0.536	0.535, 0.538	0.011	0.9	1.0
	TC	0.258	0.265, 0.229	1.635	0.2	0.5
	TT	0.162	0.156, 0.186	1.645	0.2	0.5
	GC	0.045	0.044, 0.047	0.028	0.8	1.0
rs1155563_rs2298849	TT	0.524	0.516, 0.558	1.676	0.2	0.5
	CT	0.275	0.280, 0.256	0.667	0.4	0.8
	TC	0.173	0.175, 0.167	0.104	0.7	1.0
	CC	0.027	0.029, 0.019	0.956	0.3	0.7
rs7041_rs1155563_rs2298849	GTT	0.397	0.396, 0.403	0.042	0.8	1.0
	TCT	0.235	0.240, 0.215	0.846	0.4	0.9
	GTC	0.138	0.139, 0.136	0.02	0.9	1.0
	TTT	0.127	0.120, 0.155	2.68	0.1	0.4
	GCT	0.040	0.040, 0.042	0.021	0.9	1.0
	TTC	0.035	0.036, 0.032	0.149	0.7	1.0
	TCC	0.023	0.025, 0.014	1.279	0.3	0.7

HD women vs. HD men

Polymorphisms	Haplotypes	Frequency	Case, control ratios	χ^2	*P* value	P_{corr} value[a]
rs7041_rs1155563	GT	0.537	0.538, 0.536	0.011	0.9	1.0
	TC	0.268	0.271, 0.265	0.1	0.8	1.0
	TT	0.148	0.137, 0.155	1.341	0.2	0.6
	GC	0.048	0.054, 0.044	1.038	0.3	0.7
rs1155563_rs2298849	TT	0.510	0.501, 0.517	0.55	0.5	0.8
	CT	0.283	0.289, 0.278	0.297	0.6	0.9
	TC	0.174	0.174, 0.174	0.001	1.0	1.0
	CC	0.033	0.036, 0.031	0.397	0.5	0.9
rs7041_rs1155563_rs2298849	GTT	0.398	0.396, 0.399	0.012	0.9	1.0
	TCT	0.242	0.245, 0.240	0.084	0.8	1.0
	GTC	0.139	0.142, 0.137	0.094	0.8	1.0
	TTT	0.112	0.103, 0.118	1.071	0.3	0.9
	GCT	0.041	0.045, 0.039	0.433	0.5	1.0
	TTC	0.036	0.034, 0.037	0.2	0.7	1.0
	TCC	0.025	0.026, 0.025	0.01	0.9	1.0

a – calculated using permutation test and a total of 1000 permutations

Supplementary Table 7. Clinical and laboratory data of hemodialysis women divided according to *GC* rs2298849 polymorphic variants

Parameter	*GC* rs2298849			Odds ratio (95% CI)	*P* value
	TT	CT	CC		
clinical data, n = 390	n = 249	n = 123	n = 18		
coronary artery disease, n (% of all)	78 (31)	41 (33)	9 (50)	TT vs. CT + CC: 1.205 (0.758–1.905) TT + CT vs. CC: 2.126 (0.726–6.204) TT vs. CC: 2.192 (0.737–6.483)	0.5 0.2 0.2
myocardial infarction, n (% of all)	34 (14)	27 (22)	2 (11)	TT vs. CT + CC: 1.637 (0.910–2.925) TT + CT vs. CC: 0.637 (0.069–2.825) TT vs. CC: 0.790 (0.085–3.611)	0.1 0.8 1.0
parathyroidectomy/cinacalcet, n (% of all)	54 (22)	25 (20)	5 (28)	TT vs. CT + CC: 0.976 (0.568–1.657) TT + CT vs. CC: 1.426 (0.386–4.421) TT vs. CC: 1.389 (0.371–4.379)	1.0 0.7 0.7
laboratory data, n = 350	n = 226	n = 110	n = 14		
25(OH)D, ng/ml	11.9 (4.5–20.7) n = 56	11.8 (6.6–50.0) n = 33	11.8 (8.3–13) n = 4	TT vs. CT + CC: TT + CT vs. CC: TT vs. CC:	0.6[a] 0.5[a] 0.3[b]
total calcium, mg/dl	8.97 (6.0–11.4)	8.94 (6.8–10.8)	8.85 (7.5–10.3)	TT vs. CT + CC: TT + CT vs. CC: TT vs. CC:	0.5[a] 0.8[a] 0.7[a]
phosphates, mg/dl	5.14 (2.24–11.3)	4.78 (1.7 5–8.65)	4.92 (2.94–7.94)	TT vs. CT + CC: TT + CT *vs.* CC: TT vs. CC:	0.08[a] 0.9[a] 0.9[a]
PTH, ng/l	375 (19.5–3741)	389 (13.7–3000)	320 (48.6–1230)	TT vs. CT + CC: TT + CT vs. CC: TT vs. CC:	0.9[a] 0.4[a] 0.4[a]
total ALP, U/l	100 (25.8–1684)	98.8 (41.0–1299)	87 (41.3–331)	TT vs. CT + CC: TT + CT *vs.* CC: TT vs. CC:	1.0[a] 0.3[a] 0.3[a]

Data are presented as median and range or number and percentage.
a – Mann–Whitney test, b – *t* test
Conversion factors to SI units are as follows: for 25(OH)D, 1 ng/ml = 2.496 nmol/l; for calcium, 1 mg/dl = 0.25 mmol/l; for phosphorus, 1 mg/dl = 0.323 mmol/l
Abbreviations: ALP – alkaline phosphatase, PTH – parathyroid hormone, others – see TABLE 3

Supplementary Table 8. Clinical and laboratory data of hemodialysis women divided according to *GC* rs7041 polymorphic variants

Parameter	*GC* rs7041			Odds ratio (95% CI)	*P* value
	GG	GT	TT		
clinical data, n = 366	n = 125	n = 188	n = 53		
coronary artery disease, n (% of all)	47 (38)	58 (31)	16 (30)	GG vs. GT + TT: 0.735 (0.456–1.191) GG + GT vs. TT: 0.857 (0.424–1.666) GG vs. TT: 0.718 (0.335–1.497)	0.2 0.8 0.4
myocardial infarction, n (% of all)	23 (18)	28 (15)	7 (13)	GG vs. GT + TT: 0.754 (0.409–1.412) GG + GT vs. TT: 0.782 (0.282–1.879) GG vs. TT: 0.675 (.228–1.778)	0.4 0.7 0.5
parathyroidectomy/cinacalcet, n (% of all)	25 (20)	34 (18)	12 (23)	GG vs. GT + TT: 0.944 (0.532–1.702) GG + GT vs. TT: 1.260 (0.567–2.630) GG vs. TT: 1.171 (0.487–2.694)	0.9 0.6 0.8
laboratory data, n = 327	n = 113	n = 165	n = 49		
25(OH)D, ng/ml	12.6 (8.0–50.0) n = 32	11.8 (4.5–20.3) n = 42	11.3 (5.8–15.7) n = 17	GG vs. GT + TT: GG + GT vs. TT: GG vs. TT:	0.2[a] 0.06[a] 0.04[a,b]
total calcium, mg/dl	8.93 (6.01–10.4)	8.94 (6.80–11.4)	9.15 (6.65–10.4)	GG vs. GT + TT: GG + GT *vs* TT: GG vs. TT:	0.6[a] 0.6[c] 0.7[a]
phosphates, mg/dl	5.03 (1.75–9.33)	4.80 (2.23–11.3)	5.58 (3.40–8.97)	GG vs. GT + TT: GG + GT vs. TT: GG vs. TT:	0.2[a] 0.008[a] 0.007[a]
PTH, ng/l	331 (13.7–3741)	433 (38.4–2736)	411 (36.3–2267)	GG vs. GT + TT: GG + GT vs. TT: GG vs. TT:	0.1[a] 0.4[a] 0.2[a]
total ALP, U/l	98.4 (42.8–1684)	98.2 (25.8–1110)	104 (42.5–579)	GG vs. GT + TT: GG + GT vs. TT: GG vs. TT:	0.8[a] 0.7[a] 0.9[a]

Median and range or number and percentage are presented.
a – Mann Whitney test, b – nonsignificant after the Bonferroni correction ($P > 0.017$), c – *t* test
For conversion factors: see TABLE 7
Abbreviations: see TABLES 3 and 7

Supplementary Table 9. Clinical and laboratory data of hemodialysis women divided according to *GC* rs1155563 polymorphic variants

Parametr	***GC* rs1155563**			**Odds ratio (95% CI)**	***P* value**
	TT	CT	CC		
clinical data, n = 389	n = 178	n = 165	n = 46		
coronary artery disease, n (% of all)	55 (31)	57 (35)	16 (35)	TT vs. CT + CC: 1.183 (0.756–1.855) TT + CT vs. CC: 1.100 (0.536–2.184) TT vs. CC: 1.193 (0.558–2.475)	0.5 0.9 0.7
myocardial infarction, n (% of all)	28 (16)	26 (16)	9 (20)	TT vs. CT + CC: 1.065 (0.598–1.910) TT + CT vs. CC: 1.302 (0.522–2.949) TT vs. CC: 1.303 (0.497–3.148)	0.9 0.6 0.7
parathyroidectomy/cinacalcet, n (% of all)	41 (23)	33 (20)	10 (22)	TT vs. CT + CC: 0.855 (0.512–1.430) TT + CT vs. CC: 1.010 (0.426–2.200) TT vs. CC: 0.928 (0.378–2.120)	0.6 1.0 1.0
laboratory data, n = 349	n = 157	n = 149	n = 43		
25(OH)D, ng/ml	12.7 (5.80–50.0) n = 39	11.6 (4.51–20.3) n = 43	10.9 (7.83–16.4) n = 11	TT vs. CT + CC: TT + CT vs. CC: TT vs. CC:	0.3[a] 0.5[a] 0.4[a]
total calcium, mg/dl	9.0 (6.65–11.4)	8.9 (6.01–10.8)	9.18 (7.0–10.9)	TT vs. CT + CC: TT + CT vs. CC: TT vs. CC:	0.8[b] 0.2[a] 0.4[b]
phosphates, mg/dl	5.07 (1.75–9.33)	4.92 (2.23–11.0)	5.2 (3.19–11.3)	TT vs. CT + CC: TT + CT vs. CC: TT vs. CC:	0.7[a] 0.5[a] 0.5[a]
PTH, ng/l	375 (13.7–3741)	406 (19.5–2736)	344 (38.4–1405)	TT vs. CT + CC: TT + CT vs. CC: TT vs. CC:	1.0[a] 0.6[a] 0.6[a]
total ALP, U/l	99.5 (42.8–1684)	98.4 (25.8–1110)	100 (41.0–443)	TT vs. CT + CC: TT + CT vs. CC: TT vs. CC:	0.6[a] 0.6[a] 0.5[a]

Data are presented as median and range or number and percentage.
a – Mann–Whitney test, b – *t* test
For conversion factors: see TABLE 7
Abbreviations: see TABLES 3 and 7

Supplementary Table 10. Clinical and laboratory data of hemodialysis men divided according to *GC* rs2298849 polymorphic variants

Parameter	***GC* rs2298849**			**Odds ratio (95% CI)**	***P* value**
	TT	CT	CC		
clinical data, n = 563	n = 358	n = 176	n = 29		
coronary artery disease, n (%)	137 (38)	78 (44)	13 (45)	TT vs. CT + CC: 1.288 (0.895–1.851) TT + CT vs. CC: 1.206 (0.522–2.734) TT vs. CC: 1.311 (0.561–3.006)	0.2 0.8 0.6
myocardial infarction, n (%)	86 (24)	43 (24)	9 (31)	TT vs. CT + CC: 1.075 (0.706–1.627) TT + CT vs. CC: 1.413 (0.552–3.342) TT vs. CC: 1.423 (0.549–3.415)	0.8 0.5 0.5
parathyroidectomy/cinacalcet, n (%)	62 (17)	33 (19)	1 (3)	TT vs. CT + CC: 0.949 (0.580–1.533) TT + CT vs. CC: 0.165 (0.004–1.026) TT vs. CC: 0.171 (0.004–1.076)	0.9 0.06 0.07
laboratory data, n = 476	n = 303	n = 147	n = 26		
25(OH)D, ng/ml	15.5 ± 3.96 n = 82	15.7 ± 5.84 n = 35	13.0 ± 0.96 n = 3	TT vs. CT + CC: TT + CT vs. CC: TT vs. CC:	1.0[a] 0.3[b] 0.3[b]
total calcium, mg/dl	8.8 (6.6–12.3)	8.8 (7.2–10.6)	8.9 (6.8–10.1)	TT *vs* CT + CC: TT + CT vs. CC: TT *vs* CC:	0.6[c] 0.8[c] 0.9[c]
phosphates, mg/dl	5.1 (2.0–10.4)	4.9 (2.0–12.0)	5.1 (2.5–8.9)	TT *vs* CT + CC: TT + CT vs. CC: TT vs. CC:	0.7[c] 0.6[c] 0.6[c]
PTH, ng/l	363 (7.3–3757)	402 (73.5–2588)	301 (70.2–1500)	TT vs. CT + CC: TT + CT vs. CC: TT vs. CC:	0.2[c] 0.2[c] 0.3[c]
total ALP, U/l	92.6 (40.5–977)	87.3 (38.3–860)	98.0 (57.0–223)	TT vs. CT + CC: TT + CT vs. CC: TT vs. CC:	0.5[c] 0.7[c] 0.8[c]

Data are presented as mean and standard deviation, median and range, or number and percentage.
a – Cochran Cox test, b – *t* test, c – Mann–Whitney test
For conversion factors: see TABLE 7
Abbreviations: see TABLES 3 and 7

Supplementary Table 11. Clinical and laboratory data of hemodialysis men divided according to *GC* rs7041 polymorphic variants

Parameter	***GC* rs7041**			**Odds ratio (95% CI)**	***P* value**
	GG	GT	TT		
clinical data, n = 546	n = 183	n = 261	n = 102		
coronary artery disease, n (%)	73 (40)	106 (41)	39 (38)	GG vs. GT + TT: 1.002 (0687–1.467) GG + GT vs. TT: 0.916 (0.572–1.456) GG vs. TT: 0.933 (0.549–1.577)	1.0 0.8 0.9
myocardial infarction, n (%)	47 (26)	64 (25)	23 (23)	GG vs. GT + TT: 0.912 (0.595–1.409) GG + GT vs. TT: 0.873 (0.499–1.486) GG vs. TT: 0.842 (0.453–1.539)	0.7 0.7 0.7
parathyroidectomy/cinacalcet, n (%)	36 (20)	39 (15)	20 (20)	GG vs. GT + TT: 0.793 (0.490–1.295) GG + GT vs. TT: 1.200 (0.656–2.121) GG vs. TT: 0.996 (0.511–1.901)	0.4 0.6 1.0
laboratory data, n = 458	n = 156	n = 217	n = 85		
25(OH)D, ng/ml	16.4 ± 4.69 n = 44	15.3 ± 4.59 n = 54	14.0 ± 3.77 n = 20	GG vs. GT + TT: GG + GT vs. TT: GG vs. TT:	0.09[a] 0.1[a] 0.05[a]
total calcium, mg/dl	8.8 (6.6–10.6)	8.8 (7.3–12.3)	8.8 (7.5–11.6)	GG vs. GT + TT: GG + GT vs. TT: GG vs. TT:	0.8[b] 0.4[b] 0.7[b]
phosphates, mg/dl	5.1 (2–12)	5.0 (2.0–10.4)	5.2 (2.3–10.5)	GG vs. GT + TT: GG + GT vs. TT: GG vs. TT:	0.8[b] 0.3[b] 0.6[b]
PTH, ng/l	386 (29.5–2992)	367 (7.3–3757)	337 (12.7–2570)	GG vs. GT + TT: GG + GT vs. TT: GG vs. TT:	0.6[b] 0.8[b] 0.7[b]
total ALP, U/l	88.1 (40.5–977)	95.5 (38.3–860)	88.3 (42.5–324)	GG vs. GT + TT: GG + GT vs. TT: GG vs. TT:	0.3[b] 0.8[b] 0.7[b]

Data are presented as mean and standard deviation, median and range, or number and percentage.
a – *t* test, b – Mann–Whitney test
For conversion factors: see TABLE 7
Abbreviations: see TABLES 3 and 7

Supplementary Table 12. Clinical and laboratory data of hemodialysis men divided according to *GC* rs1155563 polymorphic variants

Parameter	***GC* rs1155563**			**Odds ratio (95% CI)**	***P* value**
	TT	CT	CC		
clinical data, n = 565	n = 277	n = 221	n = 67		
coronary artery disease, n (%)	113 (41)	90 (41)	26 (39)	TT vs. CT + CC: 0.979 (0.690–1.389) TT + CT vs. CC: 0.922 (0.523–1.599) TT vs. CC: 0.920 (0.510–1.641)	1.0 0.9 0.9
myocardial infarction, n (%)	71 (26)	52 (24)	16 (24)	TT vs. CT + CC: 0.897 (0.600–1.340) TT + CT vs. CC: 0.957 (0.491–1.780) TT vs. CC: 0.910 (0.455–1.748)	0.6 1.0 0.9
parathyroidectomy/cinacalcet, n (%)	50 (18)	34 (15)	13 (19)	TT vs. CT + CC: 0.885 (0.558–1.405) TT + CT vs. CC: 1.187 (0.568–2.326) TT vs. CC: 1.093 (0.508–2.223)	0.7 0.7 0.9
laboratory data, n = 477	n = 240	n = 181	n = 56		
25(OH)D, ng/ml	16 ± 4.68 n = 54	15.5 ± 4.43 n = 50	13.6 ± 4.13 n = 16	TT vs. CT + CC: TT + CT vs. CC: TT vs. CC:	0.2[a] 0.07[a] 0.07[a]
total calcium, mg/dl	8.8 (6.6–11.7)	8.8 (7.2–12.3)	8.7 (7.5–11.6)	TT vs. CT + CC: TT + CT vs. CC: TT vs. CC:	0.4[b] 0.2[b] 0.3[b]
phosphates, mg/dl	5.2 (2.0–12.0)	4.9 (2.4–10.3)	5.3 (2.3–8.9)	TT vs. CT + CC: TT + CT vs. CC: TT vs. CC:	0.9[b] 0.1[b] 0.3[b]
PTH, ng/l	378 (29.5–2435)	363 (7.3–3757)	392 (12.7–2992)	TT vs. CT + CC: TT + CT vs. CC: TT vs. CC:	0.8[b] 0.4[b] 0.5[b]
total ALP, U/l	93.0 (40.5–692)	95.5 (38.3–379)	82.9 (42.5–977)	TT vs. CT + CC: TT + CT vs. CC: TT vs. CC:	0.9[b] 0.4[b] 0.5[b]

Data are presented as median and range or number and percentage.
a – *t* test, b – Mann–Whitney test
For conversion factors: see TABLE 7
Abbreviations: see TABLES 3 and 7

Supplementary Table 13. Clinical and laboratory data of all hemodialysis patients divided according to *GC* rs2298849 polymorphic variants

Parameter	***GC* rs2298849**			**Odds ratio (95% CI)**	***P* value**
	TT	CT	CC		
clinical data, n = 953	n = 607	n = 299	n = 47		
coronary artery disease, n (%)	215 (35)	119 (40)	22 (47)	TT vs. CT + CC: 1.254 (0.947–1.660) TT + CT vs. CC: 1.507 (0.796–2.833) TT vs. CC: 1.604 (0.840–3.042)	0.1 0.2 0.2
myocardial infarction, n (%)	120 (20)	70 (23)	11 (23)	TT vs. CT + CC: 1.240 (0.888–1.726) TT + CT vs. CC: 1.151 (0.519–2.366) TT vs. CC: 1.240 (0.552–2.580)	0.2 0.8 0.7
parathyroidectomy/cinacalcet, n (%)	116 (19)	58 (19)	6 (13)	TT vs. CT + CC: 0.961 (0.673–1.363) TT + CT vs. CC: 0.616 (0.210–1.491) TT vs. CC: 0.619 (0.210–1.516)	0.9 0.4 0.4
laboratory data, n = 826	n = 529	n = 257	n = 40		
25(OH)D, ng/ml	14.3 (4.5–24.3) n = 138	13.2 (5.7–50) n = 68	12.4 (8.3–14) n = 7	TT vs. CT + CC: TT + CT vs. CC: TT vs. CC:	0.2[a] 0.2[a] 0.1[a]
total calcium, mg/dl	8.9 (6–12.2)	8.8 (6.8–10.8)	8.9 (6.8–10.3)	TT vs. CT + CC: TT + CT vs. CC: TT vs. CC:	0.4[a] 0.9[a] 0.8[a]
phosphates, mg/dl	5.1 (2–11.3)	4.8 (4.81.8–12)	5 (2.5–8.9)	TT vs. CT + CC: TT + CT vs. CC: TT vs. CC:	0.2[a] 0.6[a] 0.8[a]
PTH, ng/l	369.1 (7.3–3757)	394.4 (13.7–3000)	300.7 (48.6–1499.8)	TT vs. CT + CC: TT + CT vs. CC: TT vs. CC:	0.3[a] 0.1[a] 0.2[a]
total ALP, U/l	96.3 (25.8–1684)	94 (38.3–1299.3)	95.8 (41.3–331)	TT vs. CT + CC: TT + CT vs. CC: TT vs. CC:	0.6[a] 0.7[a] 0.6[a]

Data are presented as median and range or number and percentage.
a – *t* test, b – Mann–Whitney test
For conversion factors: see TABLE 7
Abbreviations: see TABLES 3 and 7

Supplementary Table 14. Clinical and laboratory data of all hemodialysis patients divided according to *GC* rs7041 polymorphic variants

Parameter	*GC* rs7041			Odds ratio (95% CI)	*P* value
	GG	GT	TT		
clinical data, n = 912	n = 308	n = 449	n = 155		
coronary artery disease, n (%)	120 (39)	164 (37)	55 (35)	GG vs. GT + TT: 0.891 (0.665–1.195) GG + GT vs. TT: 0.916 (0.626–1.331) GG vs. TT: 0.862 (0.564–1.310)	0.5 0.7 0.5
myocardial infarction, n (%)	70 (23)	92 (20)	30 (19)	GG vs. GT + TT: 0.861 (0.610–1.220) GG + GT vs. TT: 0.882 (0.550–1.379) GG vs. TT: 0.816 (0.487–1.346)	0.4 0.7 0.8
parathyroidectomy/cinacalcet, n (%)	61 (20)	73 (16)	32 (21)	GG vs. GT + TT: 0.852 (0.593–1.233) GG + GT vs. TT: 1.210 (0.759–1.887) GG vs. TT: 1.053 (0.629–1.740)	0.4 0.4 0.9
Laboratory data, n = 785	n = 269	n = 382	n = 134		
25(OH)D, ng/ml	15.9 (8.0–50.0) n = 76	13.9 (4.5–28.5) n = 96	12.7 (5.7–20.9) n = 37	GG vs. GT + TT: GG + GT vs. TT: GG vs. TT:	0.04[a,b] 0.05[a] 0.016[a]
total calcium, mg/dl	8.9 (6.0–10.6)	8.9 (6.8–12.3)	8.9 (6.7–11.6)	GG vs. GT + TT: GG + GT vs. TT: GG vs. TT:	0.6[a] 0.4[a] 0.7[a]
phosphates, mg/dl	5.1 (1.8–12)	4.9 (2.0–11.3)	5.4 (2.3–10.5)	GG vs. GT + TT: GG + GT vs. TT: GG vs. TT:	0.5[a] 0.02[a,b] 0.04[a,b]
PTH, ng/l	361.5 (13.7–3740.7)	388.2 (7.3–3757)	342.4 (12.7–2570)	GG vs. GT + TT: GG + GT vs. TT: GG vs. TT:	0.6[a] 0.8[a] 0.7[a]
total ALP, U/l	91.5 (40.5–1684)	96.5 (25.8–1109.5)	98.3 (42.5–579)	GG vs. GT + TT: GG + GT vs. TT: GG vs. TT:	0.6[a] 0.8[a] 0.7[a]

Data are presented as median and range or number and percentage.
a – Mann–Whitney test, b – nonsignificant after the Bonferroni correction ($P > 0.017$), c – *t* test
For conversion factors: see TABLE 7
Abbreviations: see TABLES 3 and 7

Supplementary Table 15. Clinical and laboratory data of all hemodialysis patients divided according to *GC* rs1155563 polymorphic variants

Parameter	***GC* rs1155563**			**Odds ratio (95% CI)**	***P* value**
	TT	CT	CC		
clinical data, n = 954	n = 455	n = 386	n = 113		
coronary artery disease, n (%)	168 (37)	147 (38)	42 (37)	TT vs. CT + CC: 1.042 (0.794–1.366) TT + CT vs. CC: 0.988 (0.641–1.508) TT vs. CC: 1.011 (0.642–1.578)	0.8 1.0 1.0
myocardial infarction, n (%)	99 (22)	78 (20)	25 (22)	TT vs. CT + CC: 0.935 (0.677–1.292) TT + CT vs. CC: 1.066 (0.635–1.738) TT vs. CC: 1.022 (0.594–1.711)	0.7 0.9 1.0
parathyroidectomy/cinacalcet, n (%)	91 (20)	67 (17)	23 (20)	TT vs. CT + CC: 0.880 (0.628–1.233) TT + CT vs. CC: 1.105 (0.645–1.829) TT vs. CC: 1.022 (0.583–1.740)	0.5 0.7 1.0
laboratory data, n = 826	n = 397	n = 330	n = 99		
25(OH)D, ng/ml	14.7 (5.1–50) n = 93	13.6 (4.5–28.5) n = 93	13.4 (5.7–20.9) n = 27	TT vs. CT + CC: TT + CT vs. CC: TT vs. CC:	0.06[a] 0.09[a] 0.05[a]
total calcium, mg/dl	8.9 (6.6–11.7)	8.9 (6–12.3)	8.9 (7–11.6)	TT vs. CT + CC: TT + CT vs. CC: TT vs. CC:	0.9[a] 0.08[a] 0.04[c,b]
phosphates, mg/dl	5.1 (1.8–12)	4.9 (2.2–11)	5.3 (2.3–11.3)	TT vs. CT + CC: TT + CT vs. CC: TT vs. CC:	0.9[a] 0.09[a] 0.2[a]
PTH, ng/l	376 (13.7–3740.7)	378.9 (7.3–3757)	385.7 (12.7–2991.5)	TT vs. CT + CC: TT + CT vs. CC: TT vs. CC:	0.8[a] 0.8[a] 0.9[a]
total ALP, U/l	95.4 (40.5–1684)	97 (25.8–1109.5)	89.3 (41–977.3)	TT vs. CT + CC: TT + CT vs. CC: TT vs. CC:	1.0[a] 0.3[a] 0.3[a]

Data are presented as median and range or number and percentage.
a – Mann–Whitney test, b – nonsignificant after the Bonferroni correction ($P > 0.017$), c – *t* test
For conversion factors: see TABLE 7
Abbreviations: see TABLES 3 and 7

Supplementary Figure 1. The Linkage disequilibrium (LD) plot of HapMap single nucleotide polymorphisms (SNPs) within the *GC* region. The plot was generated using the genotype data from HapMap CEU samples (minimum minor allele frequency, 0.2) and the Haploview 4.0 software (Broad Institute, Cambridge, Massachusetts, United States). The names of the examined SNPs are enclosed in boxes. A. In the D' value plot, numbers represents D' values expressed as a percentage of maximal value (1.0). Squares without numbers correspond to D' = 1.0. A red-to-white gradient shows highest (1.0) to lowest (0.0) D' value. B. In the r^2 value plot, numbers represents r^2 values expressed as a percentage of maximal value (1.0). Squares without numbers correspond to r^2 = 1.0. A black-to-white gradient shows highest (1.0) to lowest (0.0) r^2 value.