

# CLINICAL ASPECTS OF VITAMIN D PATHWAY GENE POLYMORPHISMS IN HEMODIALYSIS WOMEN AND MEN

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## Methods:

## Background/Objectives:

Vitamin D is casually associated with life-threatening diseases like myocardial infarction, cerebral stroke, and bone mineral disorders. Prevalence of these diseases is gender-dependent.

Our aim was to evaluate a frequency distribution of vitamin D pathway gene polymorphisms in hemodialysis (HD) women and men in relation to prevalence of coronary artery disease (CAD) and severity of secondary hyperparathyroidism (sHPT).

HD women (n = 431, age 63.4 + 14.8 years) and men (n = 625, age 61.3 + 14.8 years) as well as control women (n = 163, age 49.5 + 12.7 years) and men (n = 150, age 48.9 + 12.4 years) were enrolled into the study.

Polymorphisms of genes encoding vitamin D binding protein (GC): rs2298849 (genotyped by High Resolution Melt analysis - HRM), rs7041 (Restriction Fragment Length Polymorphism analysis - RFLP, restriction enzyme - HaeIII), rs115563 (HRM), vitamin D receptor (VDR): rs2228570 (RFLP, FokI), rs1544410 (RFLP, FspI), and retinoid x receptor A (RXRA): rs10776909 (HRM), rs10881578 (HRM), rs749759 (RFLP, BstXI) were genotyping in all study subjects. Frequency distributions of the respective genotypes were compared between groups with and without age matching.

Clinical data [prevalence of CAD, myocardial infarction (MI), parathyroidectomy (PTX), and treatment of cinacalcet (TC), 955 patients reviewed for each parameter] and laboratory results [serum Ca, P, ALP, and PTH, 827 mean values of 2-4 results for each parameter; plasma 25(OH)D, 210 determinations] were compared in HD women and men bearing different polymorphic variants of the analyzed genotypes. In patients who underwent PTX or TC, laboratory data shown before these treatment commencements were taken into analysis.

Patient characteristics

Parameter	HD women	HD men	P value
Demographic data			
Age, years	63.4 ± 14.8	61.3 ± 14.8	0.507*
RRT duration, years	2.96 (0.26–26.1)	2.46 (0.07–20.0)	0.314*
Main causes of end-stage renal disease			
Diabetic nephropathy	126 (29.2)	176 (28.3)	0.729*
Hypertensive nephropathy	72 (16.7)	119 (19.1)	0.371*
Chronic glomerulonephritis	56 (13.0)	96 (15.5)	0.286*
Chronic interstitial nephritis	46 (11.1)	83 (13.4)	0.759*
Clinical data			
Coronary artery disease	128 (30.3)	229 (41.1)	0.017*
Myocardial infarction	63 (14.6)	139 (22.5)	0.002*
PTX, n (%)	16 (0.4)	12 (0.2)	0.082*
TC, n (%)	98 (0.17)	85 (0.15)	0.325*
PTX and/or TC, n (%)	94 (0.22)	97 (0.17)	0.094*
Laboratory data			
25(OH)D (ng/mL)	11.8 (4.51–30.0)	15.5 (5.10–30.1)	0.000003*
Total calcium (mg/dL)	8.94 ± 0.77	8.84 ± 0.76	0.009*
Phosphorus (mg/dL)	5.03 (1.75–17.41)	5.06 (1.95–12.0)	0.903*
PTH (pg/mL)	365 (13.7–3,741)	376 (7.3–3,757)	0.896*
Total ALP (U/L)	99.1 (25.8–1,684)	92.3 (36.3–977)	0.003*
ALP (U/L)	13 (0.6–209)	14 (2–195)	0.002*
ASP (U/L)	15 (5–106)	15 (5–177)	0.351*
GGT (U/L)	25 (4–441)	29 (1–662)	0.0001*
Total ALP (U/L) with patients having ALT, ASP or GGT excluded*	98.3 (25.8–1,299)	86.5 (40.5–977)	0.0004*

Table 1.

RXRA	HD women (frequency)	Control women (frequency)	Odds ratio (95% CI)	Two-tailed p	P <sub>gender</sub>	P <sub>interaction</sub>	Power (%)	P for deviation from HWE and expected genotype frequencies	
								HD women	Control women
rs10776909	n=425	n=163						0.706	0.531
CC	262 (61.6)	115 (70.7)	Referent*	-	0.026	0.081		263 (61.6)	115 (70.7)
CT	149 (35.3)	44 (27.0)	1.486 (0.980-2.276)	0.063				146 (35.4)	44 (27.1)
TT	19 (0.04)	4 (0.02)	2.085 (0.671-8.601)	0.268				20 (0.05)	4 (0.02)
CT+TT	168 (39.3)	48 (29.2)	1.536 (1.026-2.319)	0.036				167 (39.4)	48 (29.2)
MAF	187 (0.22)	52 (0.16)	1.466 (1.038-2.102)	0.029				187 (0.22)	52 (0.16)
rs10881578	n=428	n=163						0.527	0.876
AA	225 (53.0)	88 (54.0)	Referent*	-	0.675	0.901		222 (52.8)	88 (54.0)
AG	167 (39.3)	63 (39.0)	1.037 (0.897-1.197)	0.331				167 (39.3)	63 (39.0)
GG	36 (0.08)	12 (0.07)	1.173 (0.565-2.594)	0.797				33 (0.08)	12 (0.07)
AG+GG	203 (0.47)	75 (0.46)	1.059 (0.726-1.546)	0.829				197 (0.46)	75 (0.46)
MAF	239 (0.28)	87 (0.27)	1.064 (0.792-1.437)	0.729				237 (0.55)	87 (0.53)
rs749759	n=411	n=160						0.780	0.551
GG	226 (55.0)	94 (58.8)	Referent*	-	0.311	0.563		227 (55.5)	95 (60.0)
AG	159 (38.9)	59 (37.0)	1.121 (0.751-1.679)	0.629				157 (38.6)	56 (35.0)
AA	26 (0.06)	7 (0.04)	1.545 (0.625-4.360)	0.438				29 (0.07)	8 (0.05)
AG+AA	185 (0.45)	66 (0.41)	1.166 (0.793-1.720)	0.472				184 (0.45)	64 (0.40)
MAF	211 (0.26)	73 (0.23)	1.168 (0.854-1.609)	0.355				211 (0.26)	73 (0.23)

Table 2.

Parameter	RXRA rs10881578			Odds ratio (95% CI)	P value
	AA	AG	GG		
Clinical data, n = 399	n=211	n=142	n=36		
Coronary artery disease, n (%)	65 (31.0)	50 (35.2)	13 (36.1)	AA vs AG: 1.231 (0.787-1.922)	0.335
Myocardial infarction, n (%)	31 (15.2)	26 (18.3)	6 (16.7)	AA vs AG: 1.273 (0.715-2.267)	0.439
Parathyroidectomy, n (%)	7 (0.03)	7 (0.05)	2 (0.06)	AA vs AG: 1.552 (0.502-5.009)	0.544
Treatment with cinacalcet, n (%)	38 (0.18)	20 (0.14)	10 (0.28)	AA vs AG: 1.424 (0.151-6.814)	0.893
Parathyroidectomy/cinacalcet, n (%)	45 (0.21)	27 (0.19)	12 (0.33)	AA vs AG: 1.035 (0.618-1.728)	0.986
Laboratory data, n = 359	n=196	n=130	n=34		
25(OH)D (ng/mL)	11.9 (5.8-50)	11.9 (7.8-19)	11.2 (4.51-18.4)	AA vs AG: 1.161 (0.365-3.580)	0.823*
Total calcium (mg/dL)	8.95 (0.10-9)	8.98 (7-11.4)	8.87 (7-9.9)	AA vs AG: 1.023 (0.524-1.614)	0.971
Phosphorus (mg/dL)	5.2 (2-10.5)	4.8 (1.8-11.3)	5.3 (2-8.7)	AA vs AG: 1.056 (0.796-1.465)	0.159
PTH (pg/mL)	432.6 (19.5-3000)	306.4 (13.7-3740.7)	387.98 (45.2-2266.7)	AA vs AG: 1.035 (0.618-1.728)	0.986
PTH < 150 pg/mL, n (%)	37 (0.20)	28 (0.22)	6 (0.18)	AA vs AG: 1.053 (0.623-1.835)	0.856
PTH > 500 pg/mL, n (%)	86 (0.49)	41 (0.32)	15 (0.44)	AA vs AG: 0.863 (0.272-2.388)	0.973
Total ALP (U/L)	103.5 (25.8-1299.3)	94.5 (41.3-1684)	99.4 (49-500.8)	AA vs AG: 1.175 (0.534-2.541)	0.789

Table 3.

Parameter	VDR rs2228570			Odds ratio (95% CI)	P value
	CC	CT	TT		
Clinical data, n = 351	n=105	n=176	n=80		
Coronary artery disease, n (%)	31 (30.0)	59 (33.4)	29 (36.3)	CC vs CT: 1.250 (0.749-2.124)	0.415
Myocardial infarction, n (%)	16 (15.7)	28 (16.0)	14 (17.5)	CC vs CT: 1.092 (0.596-2.183)	0.919
Parathyroidectomy, n (%)	5 (0.05)	9 (0.05)	2 (0.03)	CC vs CT: 1.143 (0.542-2.525)	0.807
Treatment with cinacalcet, n (%)	19 (0.18)	31 (0.18)	13 (0.16)	CC vs CT: 0.939 (0.503-1.606)	0.945
Parathyroidectomy/cinacalcet, n (%)	14 (0.13)	40 (0.23)	15 (0.19)	CC vs CT: 1.179 (0.916-1.521)	0.095
Laboratory data, n = 321	n=93	n=156	n=70		
25(OH)D (ng/mL)	14.2 (8.1-20.7)	11.4 (4.51-18.2)	12.8 (5.8-50)	CC vs CT: 1.107 (0.689-2.007)	0.662
Total calcium (mg/dL)	8.89 ± 0.75	8.92 ± 0.82	9.07 ± 0.83	CC vs CT: 1.088 (0.729-1.630)	0.369*
Phosphorus (mg/dL)	4.79 (2.23-8.53)	5.1 (7.5-11.27)	4.89 (2.48-10.96)	CC vs CT: 1.086 (0.621-1.900)	0.894
PTH (pg/mL)	406.25 (21.47-3000)	416 (13.72-3740.67)	359.74 (43.41-3755.5)	CC vs CT: 0.988 (0.370-2.031)	0.989
PTH < 150 pg/mL, n (%)	16 (0.17)	28 (0.18)	16 (0.23)	CC vs CT: 1.151 (0.593-2.322)	0.791
PTH > 500 pg/mL, n (%)	38 (0.41)	69 (0.44)	28 (0.37)	CC vs CT: 1.034 (0.616-1.744)	0.996
Total ALP (U/L)	96 (42.5-1299.3)	99.75 (25.75-1684)	101 (44.75-1109.5)	CC vs CT: 1.070 (0.477-1.894)	0.100

Table 4.

Parameter	RXRA rs10776909			Odds ratio (95% CI)	P value
	CC	CT	TT		
Clinical data, n = 388	n=235	n=134	n=19		
Coronary artery disease, n (%)	75 (32.0)	40 (30.0)	12 (63.0)	CC vs CT: 1.098 (0.695-1.730)	0.732
Myocardial infarction, n (%)	32 (0.14)	24 (0.18)	6 (0.32)	CC vs CT: 1.547 (0.811-2.770)	0.154
Parathyroidectomy, n (%)	11 (0.05)	5 (0.04)	0 (0.00)	CC vs CT: 2.928 (0.848-8.897)	0.092
Treatment with cinacalcet, n (%)	47 (0.20)	21 (0.16)	0 (0.00)	CC vs CT: 0.608 (0.184-2.204)	0.581
Parathyroidectomy/cinacalcet, n (%)	58 (0.25)	26 (0.19)	0 (0.00)	CC vs CT: 0.625 (0.357-1.073)	0.033
Laboratory data, n = 349	n=213	n=119	n=17		
25(OH)D (ng/mL)	11.65 (5.8-50)	12.3 (6.8-18.9)	13.6 (4.51-18.4)	CC vs CT: 1.000 (0.000-0.899)	0.912
Total calcium (mg/dL)	8.9 (6.7-11.4)	9.0 (6.8-10.9)	9.07 (7-10.8)	CC vs CT: 1.000 (0.000-0.899)	0.835
Phosphorus (mg/dL)	5.1 (2.2-11.3)	5.0 (2.5-9.0)	4.5 (1.8-9.3)	CC vs CT: 1.000 (0.000-0.899)	0.912
PTH (pg/mL)	413 (19.5-3740.7)	373 (38.4-2266.7)	236.3 (13.7-421.1)	CC vs CT: 1.000 (0.000-0.899)	0.912
PTH < 150 pg/mL, n (%)	46 (0.22)	21 (0.18)	4 (0.24)	CC vs CT: 1.000 (0.000-0.899)	0.912
PTH > 500 pg/mL, n (%)	94 (0.44)	44 (0.37)	4 (0.24)	CC vs CT: 1.000 (0.000-0.899)	0.912
Total ALP (U/L)	102 (25.8-1684)	96.3 (41.3-579)	116.4 (64.8-241)	CC vs CT: 1.000 (0.000-0.899)	0.912

Table 5.

Parameter	RXRA rs10881578			Odds ratio (95% CI)	P value
	AA	AG	GG		
Clinical data, n = 389	n=211	n=142	n=36		
Coronary artery disease, n (%)	65 (31.0)	50 (35.2)	13 (36.1)	AA vs AG: 1.231 (0.787-1.922)	0.335
Myocardial infarction, n (%)	31 (15.2)	26 (18.3)	6 (16.7)	AA vs AG: 1.273 (0.715-2.267)	0.439
Parathyroidectomy, n (%)	7 (0.03)	7 (0.05)	2 (0.06)	AA vs AG: 1.552 (0.502-5.009)	0.544
Treatment with cinacalcet, n (%)	38 (0.18)	20 (0.14)	10 (0.28)	AA vs AG: 1.424 (0.151-6.814)	0.893
Parathyroidectomy/cinacalcet, n (%)	45 (0.21)	27 (0.19)	12 (0.33)	AA vs AG: 1.035 (0.618-1.728)	0.986
Laboratory data, n = 359	n=196	n=130	n=34		
25(OH)D (ng/mL)	11.9 (5.8-50)	11.9 (7.8-19)	11.2 (4.51-18.4)	AA vs AG: 1.161 (0.365-3.580)	0.823*
Total calcium (mg/dL)	8.95 (0.10-9)	8.98 (7-11.4)	8.87 (7-9.9)	AA vs AG: 1.023 (0.524-1.614)	0.971
Phosphorus (mg/dL)	5.2 (2-10.5)	4.8 (1.8-11.3)	5.3 (2-8.7)	AA vs AG: 1.056 (0.796-1.465)	0.159
PTH (pg/mL)	432.6 (19.5-3000)	306.4 (13.7-3740.7)	387.98 (45.2-2266.7)	AA vs AG: 1.035 (0.618-1.728)	0.986
PTH < 150 pg/mL, n (%)	37 (0.20)	28 (0.22)	6 (0.18)	AA vs AG: 1.053 (0.623-1.835)	0.856
PTH > 500 pg/mL, n (%)	86 (0.49)	41 (0.32)	15 (0.44)	AA vs AG: 0.863 (0.272-2.388)	0.973
Total ALP (U/L)	103.5 (25.8-1299.3)	94.5 (41.3-1684)	99.4 (49-500.8)	AA vs AG: 1.175 (0.534-2.541)	0.789

Table 6.

Parameter	VDR rs115563			Odds ratio (95% CI)	P value
	GG	AG	AA		
Clinical data, n = 556	n=206	n=262	n=88		
Coronary artery disease, n (%)	64 (0.41)	108 (0.40)	35 (0.40)	GG vs AG: 1.080 (0.680-1.714)	0.707
Myocardial infarction, n (%)	46 (0.22)	68 (0.26)	23 (0.26)	GG vs AG: 1.222 (0.801-1.880)	0.336
Parathyroidectomy, n (%)	5 (0.02)	5 (0.02)	2 (0.02)	GG vs AG: 1.065 (0.112-5.325)	1.000
Treatment with cinacalcet, n (%)	26 (0.13)	40 (0.15)	18 (0.20)	GG vs AG: 1.065 (0.112-5.325)	1.000
Parathyroidectomy/cinacalcet, n (%)	31 (0.15)	45 (0.17)	20 (0.23)	GG vs AG: 1.065 (0.112-5.325)	1.000
Laboratory data, n = 468	n=171	n=223	n=74		
25(OH)D (ng/mL)	15.55 (6.30-11)	16.65 (6.22-9)	11.65 (5.1-24.9)	GG vs AG: 1.157 (0.781-2.184)	0.345
Total calcium (mg/dL)	8.77 (7.15-12.25)	8.83 (6.6-10.55)	8.74 (7.25-11.7)	GG vs AG: 1.027 (0.781-2.184)	0.190
Phosphorus (mg/dL)	5.08 (1.95-10.2)	5.07 (2.03-10.45)	5.07 (2.49-11.99)	GG vs AG: 1.027 (0.781-2.184)	0.190
PTH (pg/mL)	379 (7.33-3757)	340.25 (29.53-2588)	425.38 (16.76-1847)	GG vs AG: 1.027 (0.781-2.184)	0.190
PTH < 150 pg/mL, n (%)	21 (0.12)	37 (0.17)	12 (0.16)	GG vs AG: 1.027 (0.781-2.184)	0.190
PTH > 500 pg/mL, n (%)	66 (0.39)	80 (0.36)	31 (0.42)	GG vs AG: 1.027 (0.781-2.184)	0.190
Total ALP (U/L)	95.8 (38.3-977.3)	91.65 (42.5-1092)	80.38 (40.5-329.5)	GG vs AG: 1.027 (0.781-2.184)	0.190

Table 7.

Parameter	VDR rs2228570			Odds ratio (95% CI)	P value
	CC	CT	TT		
Clinical data, n = 543	n=145	n=292	n=106		
Coronary artery disease, n (%)	64 (0.44)	116 (0.40)	39 (36.0)	CC vs CT: 1.079 (0.634-1.880)	0.296
Myocardial infarction, n (%)	38 (0.28)	69 (0.24)	25 (0.24)	CC vs CT: 1.079 (0.634-1.880)	0.296
Parathyroidectomy, n (%)	3 (0.02)	6 (0.02)	3 (0.03)	CC vs CT: 1.079 (0.634-1.880)	0.296
Treatment with cinacalcet, n (%)	21 (0.14)	44 (0.15)	18 (0.17)	CC vs CT: 1.079 (0.634-1.880)	0.296
Parathyroidectomy/cinacalcet, n (%)	24 (0.17)	50 (0.17)	21 (0.20)	CC vs CT: 1.079 (0.634-1.880)	0.296
Laboratory data, n = 455	n=120	n=241	n=94		
25(OH)D (ng/mL)	15.34 ± 4.54	15.56 ± 4.43	15.65 ± 5.25	CC vs CT: 1.079 (0.634-1.880)	0.809*
Total calcium (mg/dL)	8.92 (7.5-10.58)	8.72 (6.8			