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A Pilot Study of the Association *VDR* Polymorphisms With Primary Hyperparathyroidism

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Abstract. *Background/Aim: Primary hyperparathyroidism (PHPT) is the third most common endocrine disorder characterized by autonomous parathyroid hormone (PTH) production from one or more parathyroid glands and hypocalcemia. Vitamin D through its receptor is a principal regulator of parathyroid glands function. VDR gene polymorphisms, which affect the expression or structure of VDR protein, may be involved in the genetic pathogenesis of PHPT. The aim of this study was to investigate the role of FokI, ApaI, TaqI, and BsmI VDR gene polymorphisms as genetic predisposing factors for PHPT. Patients and Methods: Fifty unrelated patients with sporadic PHPT and an equal number of corresponding ethnicity, sex and age range healthy volunteers were enrolled in the study. Genotyping was performed with polymerase chain reaction and restriction fragment length polymorphism assay. Results: Statistically significant difference was observed in TaqI genotype distribution between PHPT patients and controls, while no association was detected for the other studied polymorphisms. Conclusion: TaqI TT and TC genotypes may be associated with PHPT risk in Greek population. Further independent studies are needed to replicate and validate the role of VDR TaqI polymorphism in PHPT predisposition.*

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Parathyroid hormone (PTH) and calcitriol (1,25-dihydroxyvitamin D3) are the two principal hormones regulating calcium homeostasis in the human body. PTH is secreted by the parathyroid glands at low calcium serum levels and restores calcium levels by increasing bone resorption, enhancing calcium reabsorption in the kidneys, and stimulating the synthesis of calcitriol. Subsequently, calcitriol provides a negative feedback signal to the parathyroid glands and represses transcription of the *PTH* gene, discontinuing the release of PTH. Calcitriol exerts its function through the vitamin D receptor (VDR), a member of the steroid hormone receptor superfamily. After binding to VDR, calcitriol promotes phosphorylation of VDR and heterodimerization with retinoid-X receptor (RXR). Then, the complex migrates to the nucleus and binds to vitamin D response element (VDRE) in the promoter region of target genes (1, 2).

Primary hyperparathyroidism (PHPT) is an endocrine disorder characterized by hypercalcemia and autonomous secretion of parathyroid hormone (PTH) from one or more parathyroid gland(s). It is the third most common endocrine disorder that affects 0.3% of the general population and 1%-3% of postmenopausal women, and the prevalence increases with age (3, 4). The vast majority of cases (90-95%) are sporadic, the female to male ratio is about 3.3:1 and it seems that women and men present differences in age, symptoms, surgery indications, and preoperative laboratory values (5, 6). PHPT is caused from a single parathyroid adenoma in most patients (85%) and rarely from multiglandular

parathyroid hyperplasia (<15%) and parathyroid carcinoma (<1%) (7). Ionizing radiation, especially in childhood and chronic lithium use have been proposed as risk factors for sporadic PHPT (3) .

The genetic pathogenesis of sporadic PHPT is unclear. A number of genes have been reported to be involved in the disease, including cell regulatory, apoptotic and growth factor genes and genes of the Wnt/β-catenin pathway (7). Considering the high levels of PTH in PHPT patients, genes that regulate PTH secretion could also have a role in PHPT pathogenesis. Studies have shown that calcitriol *via* its receptor represses transcription of *PTH* gene and inhibits parathyroid cell proliferation (1, 8, 9). VDR expression levels have been reported to be reduced in parathyroid adenomas, and therefore may be important in parathyroid tumorigenesis (9).

The aim of this study was to investigate the potential role of *VDR* gene polymorphisms as genetic risk factors for PHPT in the Greek population. Four *VDR* gene variants (12q13-12q14) that may affect the expression and function of VDR protein were included in the present study. Specifically, the rs2228570 C/T (FokI) in exon 2, the rs731236 T/C (TaqI) in exon 9, and the rs1544410 A/G (BsmI) and rs7975232 T/G (ApaI) in intron 8 (10).

Patients and Methods

Fifty unrelated patients with PHPT (2 males and 48 females, 56.1±13.9 years) were enrolled in the study. The diagnosis of PHPT was made by the elevated PTH and calcium levels in blood serum and established with at least two imaging methods (sonography, ^{99m}Tc-sestamibi scintigraphy, 4D-CT). The PHPT of samples was also confirmed by histological examination (11). In addition, 50 ethnic matching healthy volunteers (8 males and 42 females, 50.6±18.4 years) with no personal or family history of chronic autoimmune or neoplastic diseases were studied. The sample size for this pilot study was calculated having an upper 90% confidence level and probability at 0.05 (12). The study protocol was approved by the Ethics Committees of Aristotle University of Thessaloniki. A written informed consent was obtained from each patient.

Genomic DNA was extracted from peripheral blood lymphocytes using the PureLink Genomic DNA Kit (Invitrogen, Karlsruhe, Germany). *VDR* FokI, ApaI, TaqI and BsmI polymorphisms were studied using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay, as described previously (13). All samples were run twice. The labels F, A, T, and B of the studied polymorphisms correspond to the uncut nucleotides (FokI: C, ApaI: T, TaqI: T, BsmI: A) and the f, a, t, and b to the cut ones (FokI: T, ApaI: G, TaqI: C, BsmI: G).

Pearson's chi-square test was used to test differences in polymorphism distribution between PHPT patients and controls under the six models of genetic association, which are homozygote, heterozygote, dominant, recessive, allelic, and additive. When expected values were less than 5, Fisher's exact test was used instead. Furthermore, the odds ratio (OR) with a confidence interval (CI) of 95% was calculated (reference allele *vs*. variant allele). Pearson's chi-square test was also used to examine possible deviation of genotype distributions from the Hardy–Weinberg equilibrium (HWE) in the control group and in the mixed patient/control group for each studied variant (14). A difference at $p \leq 0.05$ was considered as statistically significant in all statistical tests. All the analyses were performed using SPSS statistical package (IBM Corp., Armonk, NY, USA).

Results

The distribution of genotypes of FokI, ApaI, and BsmI polymorphisms did not deviate from HWE in the control group and in the mixed patient/control group. TaqI polymorphism was not in HWE in the control group (p=0.007). However, when we tested HWE in the mixed control-patient group, as proposed by Wang and Shete (14) for common diseases, no deviation from HWE was observed. The results of all genetic association analyses of the studied variants with PHPT are shown in Table I.

TaqI TT and TC genotypes were found to have a predisposing effect on PHPT risk according to the additive and recessive models. The distribution of the other studied polymorphisms FokI, ApaI, and BsmI did not differ significantly between PHPT patients and controls (Table I). When the studied polymorphisms were analyzed in pairs for their association with PHPT risk, statistical significance in genotype distributions between patients and controls remained for the BsmI-TaqI, FokI-TaqI, and ApaI-TaqI pairs (Table II). No statistically significant differences were found between the patient and control groups regarding the other pairs of polymorphisms (data not shown).

Discussion

PHPT is an endocrine disorder characterized by increased serum PTH and calcium concentration. Genes involved in PTH secretion may have a role in the genetic basis of the disease. Calcitriol *via* its receptor (VDR) is involved in the inhibition of both PTH secretion and parathyroid cell proliferation (8, 9). Reduced parathyroid VDR expression or function has been implicated in the pathogenesis of PHPT (9, 15).

The present study focused on the role of four common *VDR* gene polymorphisms (FokI, ApaI, TaqI, and BsmI) as candidate genetic risk factors for PHPT. These variants are potentially functional and have been extensively reported in literature for their implication in many autoimmune diseases and cancer types (16, 17). FokI polymorphism leads to a shortened, but more active, VDR protein due to an alteration in the start codon (16, 18). TaqI polymorphism is located at the 3' UTR of *VDR* gene and studies have shown that this variant regulates the stability of VDR mRNA (16, 19). ApaI and BsmI polymorphisms are intronic and although they do not alter the structure of VDR protein, they may influence gene expression by modulating mRNA stability or affecting mRNA splicing (18, 19).

Our results demonstrated a predisposing role of TaqI TT and TC genotypes in PHPT risk. This is in agreement with a

Table I. *Statistical analysis of FokI, ApaI, TaqI, and BsmI polymorphisms between patients with primary hyperparathyroidism and controls.*

SNP genotypes	Patients	Controls	Analysis model*	OR (95%CI)	*p*-Value	HWE in control group/in mixed control-patient group (13) (*p*-Value)
FokI (rs2228570)						
FF	27	19	Additive (FF *vs*. Ff *vs*. ff)		0.4	0.19/0.32
Ff	20	27				
ff	3	4				
FF	27	19	Homozygous (ff *vs*. FF)	0.53 (0.11-2.63)	0.69	
ff	3	4				
FF	27	19	Heterozygous (Ff *vs*. FF)	0.52 (0.23-1.19)	0.12	
Ff	20	27				
FF	27	19	Dominant (Ff+ff *vs*. FF)	0.52 (0.24-1.16)	0.11	
Ff+ff	23	31				
Ff+FF	47	46	Recessive (ff *vs*. Ff+FF)	0.73 (0.16-3.46)	1	
ff	3	4				
F	74	65	Allelic (f *vs*. F)	0.65 (0.36-1.2)	0.17	
f	26	35				
ApaI (rs79752)						
AA	17	17	Additive (AA *vs*. Aa *vs*. aa)		0.37	0.45/0.93
Aa	27	22				
aa	6	11				
AA	17	17	Homozygous (aa *vs*. AA)	0.55 (0.16-1.81)	0.32	
aa	6	11				
AA	17	17	Heterozygous (Aa *vs*. AA)	1.23 (0.51-2.95)	0.65	
Aa	27	22				
AA	17	17	Dominant (Aa+aa *vs*. AA)	1 (0.44-2.29)	1	
Aa+aa	33	33				
Aa+AA	44	39	Recessive (aa *vs*. Aa+AA)	0.48 (0.16-1.43)	0.18	
aa	6	11				
A	61	56	Allelic (a *vs*. A)	0.81 (0.46-1.43)	0.48	
a	39	44				
TaqI (rs731236)						
TT	18	22	Additive (TT *vs*. Tt *vs*. tt)		**0.02**	0.007/0.24
Tt	27	15				
tt	5	13				
TT	18	22	Homozygous (tt *vs*. TT)	0.47 (0.14-1.57)	0.21	
tt	5	13				
TT	18	22	Heterozygous (Tt *vs*. TT)	2.2 (0.9-5.34)	0.08	
Tt	27	15				
TT	18	22	Dominant (Tt+tt *vs*. TT)	1.4 (0.63-3.12)	0.41	
Tt+tt	32	28				
Tt+TT	45	37	Recessive (tt *vs*. Tt+TT)	0.32 (0.1-0.97)	**0.04**	
tt	5	13				
T	63	59	Allelic (t *vs*. T)	0.85 (0.48-1.5)	0.56	
t	37	41				
BsmI (rs1544410)						
BB	12	12	Additive (BB *vs*. Bb *vs*. bb)		0.61	0.78/0.69
Bb	28	24				
bb	10	14				
BB	12	12	Homozygous (bb *vs*. BB)	0.71 (0.23-2.23)	0.56	
bb	10	14				
BB	12	12	Heterozygous (Bb *vs*. BB)	1.16 (0.44-3.07)	0.75	
Bb	28	24				
BB	12	12	Dominant (Bb+bb *vs*. BB)	1 (0.4-2.5)	1	
Bb+bb	38	38				
Bb+BB	40	36	Recessive (bb *vs*. Bb+BB)	0.64 (0.25-1.62)	0.35	
bb	10	14				
B	52	48	Allelic (b *vs*. B)	0.85 (0.49-1.48)	0.57	
b	48	52				

*For all the studied polymorphisms the labels F, A, T, and B correspond to the uncut nucleotides (FokI: C, ApaI: T, TaqI: T BsmI: A) and the f, a, t, and b to the cut ones (FokI: T, ApaI: G, TaqI: C, BsmI: G). OR: Odds ratio; 95%CI: 95% confidence interval; HWE: Hardy–Weinberg equilibrium. Statistically significant *p*-values are shown in bold.

Table II. *Genotype distributions between patients and controls for the FokI-TaqI, ApaI-TaqI and BsmI-TaqI polymorphisms' pairs.*

Polymorphism pair	Genotypes combination*	Patients	Controls	*p*-Value
FokI_TaqI	FF_tt	4	4	0.04
	FF_Tt	10	6	
	FF_TT	13	9	
	Ff_tt	1	8	
	Ff_Tt	14	7	
	Ff_TT	5	12	
	ff_tt	0	1	
	ff_Tt	3	2	
	ff_TT	0	1	
ApaI_TaqI	AA_tt	5	12	0.03
	AA_Tt	12	3	
	AA_TT	0	2	
	Aa_tt	0	1	
	Aa_Tt	15	12	
	Aa_TT	12	9	
	aa_tt	0	0	
	aa_Tt	0	0	
	aa_TT	6	11	
BsmI_TaqI	BB_tt	5	10	0.04
	BB_Tt	7	1	
	BB_TT	0	2	
	Bb_tt	0	3	
	Bb_Tt	20	13	
	Bb_TT	8	8	
	bb_tt	0	0	
	bb_Tt	0	1	
	bb_TT	10	12	

*For all the studied polymorphisms, the labels F, A, T, and B correspond to the uncut nucleotides (FokI: C, ApaI: T, TaqI: T BsmI: A) and the f, a, t, and b to the cut ones (FokI: T, ApaI: G, TaqI: C, BsmI: G).

previous study, which reported overrepresentation of the TaqI T allele in postmenopausal Caucasian women with PHPT compared to controls, suggesting that this allele may be a risk factor for PHPT (20). This *VDR* polymorphism is in linkage disequilibrium with other functional variations and could affect the stability of VDR mRNA, altering VDR protein expression levels (19, 21). Homozygosity for the TaqI T allele has also been associated with lower VDR mRNA levels in PHPT patients and secondary hyperparathyroidism in hemodialysis patients (9, 22).

None of the other polymorphisms (FokI, ApaI, and BsmI) showed an association with PHPT. This is consistent with previous studies, which also failed to identify such a difference in the prevalence of these polymorphisms between PHPT patients and controls (23, 24). In contrast, three studies of Carling *et al*. identified association of G alleles of *VDR* ApaI and BsmI polymorphisms with PHPT and VDR expression levels (9, 20, 25). These contradictive results may be explained by the differences reported in the frequencies of the *VDR* alleles between populations (9, 23).

It is worth mentioning that in the present study TaqI polymorphism is in HWE in the entire sample (both patients and controls), but shows a deviation when testing the control group separately. This is in accordance with TaqI alleles' frequencies and deviation from HWE in control groups reported in other Greek (21, 26) or Caucasian populations (27-33). Additionally, a study by Wang and Shete pointed out the inappropriateness of testing HWE only in controls in case-control genetic association studies of common diseases and underlined the necessity of using a mixture sample consisted of both patients and controls (14). Moreover, departure from HWE in a human control population can be caused by natural factors, such as selective pressure against a certain genotype and, therefore, such polymorphisms should not be removed from the analysis in population-based studies (34, 35) It is worth mentioning that the association of *VDR* TaqI polymorphism with PHPT was maintained when we tested the distribution of TaqI genotypes in pairs with those of the other studied variants (FokI, ApaI, and BsmI; being in HWE in controls) between PHPT patients and controls.

To our knowledge, this is the first study to report an association of the *VDR* TaqI polymorphism with PHPT in a Greek population. However, this is a pilot study with a small sample size, therefore we emphasize the need for additional

studies in larger groups of patients of various ethnicities to replicate and validate these preliminary findings.

Conflicts of Interest

The Authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors' Contributions

A. Cho, DT, MM performed sample collection, C. Ach performed experiments and data analysis, A. Cho, C Ach wrote the manuscript, TP identified cases/controls, A. Cha designed and supervised the genetic study, A. Che examined the histopathology of PHPT samples, TP, A. Cha reviewed and edited article. All Authors contributed to the article and approved the submitted version.

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