

Vitamin D receptor gene polymorphisms in patients with thyroid cancer

Research Article

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Summary

The association between vitamin D receptor (VDR) gene polymorphisms and some diseases such as colorectal cancer, breast cancer, osteoporosis and psoriasis has been extensively investigated during the past few years. This research was performed not only because of the role of vitamin D as an anticancer agent, but also because of the suppressing action of vitamin D on TSH, the major regulator of thyroid cell growth. In this case-control study, comprising 71 thyroid cancer patients and 82 healthy population controls, we investigated the association between altered thyroid cancer risk with four polymorphisms located at the 3' end of the VDR gene detectable by ApaI, TaqI, Tru9 and BsmI restriction enzymes and with a start codon polymorphism located at the 5' end characterized by restriction enzyme FokI. The individual genetic pattern for VDR was evaluated by DNA extraction followed by PCR amplification of the VDR gene and the digestion with the restriction enzymes. For the effect of existence of the mentioned polymorphisms and thyroid cancer the odds ratio and 95% CI were calculated. All the odds ratios were within their CI, representing no relationship between these polymorphisms and risk of thyroid cancer. These observations suggest that VDR gene polymorphisms may not commonly contribute to the risk of thyroid cancer.

I. Introduction

Thyroid cancer (TC) is the most common endocrine malignancy and accounts for 1-2% of all cancers. Epidemiological studies have reported a progressive increase in the overall incidence over the past 20 years (Larijani et al, 2004). Thyroid growth is regulated by several factors, among them thyroid stimulating hormone (TSH) is a major regulator of thyroid cell growth and differentiation (Jameson and Weetman, 2005). There are some suppressor factors which attenuate the effect of TSH on thyroid cells. One of these factors is active metabolite of vitamin D (1, 25-(OH) 2-vitamin D3), which binds to vitamin D receptor (VDR), through a complex network of

genomic and non-genomic mechanisms. The TSH stimulates production of the intracellular signaling molecule 3', 5'-cyclic adenosine mono phosphate (cAMP), iodine uptake and cell growth (Berg et al, 1994; Rajendra et al, 2002). In addition, VDR is induced by P53, which is a known tumor suppressor gene in thyroid cancer (Maruyama et al, 2006).

1, 25-(OH) 2-vitamin D3 is known to have potent anti-proliferative effects in many cancer cell types, including breast and prostate cancers (Furuya et al, 1999; Watanabe et al, 1999; Habuchi et al, 2000; Bertherton-Watt et al, 2001; Cui et al, 2001; Guy et al, 2004). The anticancer properties of 1, 25-(OH) 2-vitamin D3 include

induction of differentiation and apoptosis in addition to the inhibition of cancer cell growth (Osborne et al, 2002; Rajendra et al, 2002).

1,25 (OH)₂ D₃ exerts its growth-regulatory effect through binding to the vitamin D receptor (VDR), a member of the steroid/thyroid-retinoic acid receptor family which functions as a ligand-dependent nuclear transcription factor. To date, only one VDR gene has been identified, giving rise to one main functional VDR in different tissues. Biological and immunocytochemical studies have shown that VDR is widely distributed in normal human tissues including intestine, kidney, bone, parathyroid, thyroid, skin, adrenal, breast and prostate (Malloy and Feldman, 1999). Several polymorphisms have been identified in various introns and exons of VDR gene such as ApaI, BsmI, FokI, TaqI and Tru9 (Hutchinson et al, 2000; Ye WZ et al, 2000; Ntais et al, 2003). Recent molecular and epidemiological studies have shown that these polymorphisms may be linked with many cancer risks and/or with its aggressive phenotype such as colorectal cancer (Peters et al, 2001, Wong et al, 2003), breast cancer (Curran et al, 1999; Bertherton-Watt et al, 2001; Cui et al, 2001; Guy et al, 2004) and prostate cancer (Watanabe et al, 1999; Furuya et al, 1999; Habuchi et al, 2000; Xu Y et al, 2003).

This study was conducted to investigate the association of ApaI, BsmI, FokI, TaqI and Tru9 polymorphisms of the VDR gene with thyroid cancer risk.

II. Materials and methods

A. Samples preparation

Seventy-one thyroid cancer patients were studied in a referral center for endocrine disorders. In this study, only patients with follicular and papillary thyroid cancer were included. The diagnoses were confirmed by the pathology examination from thyroid tissue samples; 58/71 patients were diagnosed as papillary type and 13/71 patients as follicular type. A total of 82 healthy volunteers, selected from the minor trauma ward with a negative history of malignancies, chemotherapy and radiotherapy were also included in this study. Cases and controls were matched according to age and sex. About 5 ml peripheral blood samples were collected from each patient. High molecular weight DNA was extracted from whole blood nuclear cells by a standard salting out procedure. (Miller et al, 1988) The concentration of DNA sample was evaluated by a UV spectrophotometer.

B. Restriction fragment length polymorphism (RFLP)

The PCR assay was performed in a final volume of 30 µl containing 50-100 ng DNA, 100 pmol of each amplification primers, 1 U Taq polymerase, and 3 µl 10X PCR buffer comprising 500 mM KCl, 0.1 M Tris HCl (pH 8.8), 25 mM MgCl₂ and 2 mM of each dNTP. PCR was carried out for 35 cycles (each cycle consisted of 30' seconds at 94°C, 30' seconds at 50-60°C (depending on the different primer sets), and 30' seconds at 72°C), with an initial denaturation at 95°C for 5 minutes.

The 825 bp fragment encompassing the BsmI polymorphic site in intron 8 was amplified using the specific primers (Table 1). When the BsmI site was present, the PCR fragment was divided into 650 and 175 bp by BsmI endonuclease digestion. The 490 bp fragment encompassing ApaI and TaqI sites was

divided into 280 and 210 bp by ApaI digestion or into 290 and 200 bp by TaqI digestion. The 265 bp fragment containing FokI site was divided into 196 and 69 by FokI digestion; and 331 bp fragment containing Tru9 was divided into 153 and 178 bp by Tru9 digestion.

The PCR products were digested overnight with BsmI (65 C), ApaI (37 C), TaqI (65 C), FokI (55 C) and Tru9 (72 C), and were electrophoresed on 2% agarose gel. The genotypes were designated as B, A, T, F, and R when the BsmI, ApaI, TaqI, FokI and Tru9 restriction sites were present respectively. All genotyping assays were performed by individuals who were unaware of the clinical status of the subjects.

C. Statistical analysis

The association between disease and genotypes were assessed by chi square and Fisher's exact tests using SPSS (version 11.5) software. The P<0.05 was considered significant. The odds ratios and their 95% confidence intervals were calculated.

The study was conducted according to the principles of the Declaration of Helsinki and was approved by the medical ethics review board of the Endocrinology and Metabolism Research Center (EMRC) of Tehran University of Medical Sciences (TUMS). A written informed consent was obtained from all patients and volunteers.

III. Results

A total of 71 patients with thyroid cancer, including 58 papillary (mean age 43.73 ± 12.81) and 13 follicular (mean age 49.69 ± 10.76) and 82 controls (mean age 43.93 ± 13.30) were included in this study. The distribution in two main histological types with respect to sex was as follows: 47 (41.2%) of papillary and 12 (10.5%) of follicular in females: 11(28.2%) of papillary and 1 (2.6%) of follicular in males.

The frequencies of ApaI, BsmI, TaqI, Tru9 and FokI genotypes, P-value and Odds ratio in cases and controls are shown in Table 2. The determination of all mentioned genotypes in patients with thyroid cancer and in the control population displayed similar frequencies and revealed no association between these polymorphisms and risk of thyroid cancer (Table 2). But about FokI polymorphism, considering FF genotype as reference group (wild type), a decreasing pattern in Odds ratio from FF to ff was seen (1.00, 0.87 and 0.37 for FF, Ff and ff respectively) (Calculated odds ratios are not shown in the Table 2). The polymorphism distribution in the present population was in Hardy-Weinberg equilibrium (P>0.05).

We also examined the allelic frequencies mentioned in polymorphisms in the patients with thyroid cancer. Except for Tru9, there were no significant differences in allelic prevalence among the cases and the controls (Table 3).

The association of VDR with thyroid cancer was analyzed with respect to sex. The distribution of genotype frequencies did not differ significantly between females with thyroid cancer and females in the control group (P-value: FokI: 0.203, BsmI: 0.536, ApaI: 0.329, TaqI: 0.804 and Tru9: 0.207). The same results were obtained for the men (P-value: FokI: 0.760, BsmI: 0.612, ApaI: 0.989, TaqI: 0.665 and Tru9: 0.179).

Because a series of these polymorphisms had been shown to be in strong linkage disequilibrium (LD) with

one another in many populations, we analyzed the presence of the LD between the BsmI, ApaI, TaqI and Tru9 polymorphisms which are located at the 3' end of VDR gene and also with FokI at the 5' end in the control group. LD was statistically significant between BsmI, ApaI and TaqI polymorphisms ($P < 0.05$) (Table 4). However, LD between Tru9 and TaqI or Tru9 and ApaI polymorphisms were not significant ($P = 0.201$). A marginal significant of LD between Tru9 and BsmI was observed (0.039). No LD was found between FokI at the 5' end and those polymorphisms located at the 3' end.

IV. Discussion

In the present study, we assessed for the first time, five VDR polymorphisms FokI, ApaI, TaqI, BsmI and Tru9 as risk factors for thyroid cancer. This became a plausible hypothesis in the light of three findings.

First: Recent studies describe the molecular basis of the anticancer activity of 1, 25 (OH) 2D3 which induces cell differentiation and inhibit proliferation, invasiveness, angiogenesis and metastatic potential (Dusso et al, 1998; Nagpal et al, 2001; Carlberg 2003; Ordonez-Moran et al, 2005). Second: 1, 25-(OH) 2-vitamin D3 at physiological concentrations inhibits both basal and TSH stimulated cAMP production in rat thyroid cells. This indicates that calcitriol may modulate the effect of TSH on thyroid function and growth (Berg et al, 1994). Third: VDR is induced by P53, which is a known tumor suppressor gene in thyroid cancer (Maruyama et al, 2006). All these findings may suggest that 1, 25-(OH) 2-vitamin D3

through binding to its receptor may act as an anticancer agent.

In our study, we see no evidence for associations between genotype defined by polymorphisms of the VDR gene and thyroid cancer susceptibility. Although, 1,25(OH)2D3 attenuated the stimulatory effects of cAMP on proliferation and iodine uptake in rat thyroid FRTL-5 cells (Berg et al, 1994), this result deserves a more comprehensive research on human thyroid cells. It is also known that mutations of the tumor suppressor, P53, appear to play an important role in the development of anaplastic thyroid cancer (Jameson and Weetman, 2005). Because we did not find enough anaplastic thyroid cancer patients, the rare variant, this type of cancer was excluded from our study.

The 3' end polymorphisms of the VDR gene do not alter the amino acid sequence (Guy et al, 2004, Taverna et al, 2005). However, It has been reported that the polymorphisms in the 3' UTR-region (BsmI, Taq I, Tru 9, Apa I and Poly-A) might alter transcriptional activity and mRNA degradation (You-Ling et al, 2005). No relationship between VDR polymorphisms located in the 3' end and the risk of thyroid cancer was found in this study. The lack of a relationship of the BsmI, TaqI and ApaI polymorphisms of VDR gene with the risk of thyroid cancer was probably due to the strong LD among these polymorphisms as observed in our study which is also reported in previous researches in different population (Morrison et al, 1992, 1994; Habuchi et al, 2000).

Table 1. The primers used in VDR gene PCR

	Primer	Ref
BsmI	F:5'-ACC TGG CCA TTG TCT CTC AC-3 R: 5'-CTA ACC AGC GGA AGA GGT CA-3	29
ApaI	F: 5'-AGC AGA GCA GAG TTC CAA GC-3' R: 5'-GTG AGG AGG GCT GCT GAG TA-3'	14
TaqI	F: 5'-AGC AGA GCA GAG TTC CAA GC-3' R: 5'-GTG AGG AGG GCT GCT GAG TA-3'	14
FokI	F: 5'-CCC TGG CAC TGA CTC TGG CTC-3' R: 5'-AAA CAC CTT GCT TCT TCT CC-3'	18,14
Tru9	F:5'-AAT ACT CAG GCT CTG CTC TT-3' R:5'-CAT CTC CAT TCC TTG AGC CT-3'	27,15

Table 2. Frequencies, P-value and Odds ratio of VDR polymorphism in patients with thyroid cancer and controls

	BsmI			ApaI			TaqI			Tru9			FokI		
	BB	Bb	bb	AA	Aa	aa	TT	Tt	tt	RR	Rr	rr	FF	Ff	ff
carcinoma	23	20	28	29	27	15	31	28	12	45	22	4	49	18	4
control	20	26	33	25	38	19	38	32	11	52	28	2	50	21	11
P-value	0.81			0.39			0.83			0.57			0.29		
Odds ratio *	1.41			1.57			0.87			0.99			0.70		
CI 95%**	0.61-2.46			0.80-3.06			0.46-1.6			0.49-2.03			0.34-1.45		

*The all Odds ratio for both capital vs capital, small and small, small were calculated (e.g. [BB vs Bb, bb])

**CI: Confidence Interval

Table 3. Allele frequency of FokI, BsmI, TaqI, Tru9 and ApaI alleles between genotypes in cases and controls

	FokI		BsmI		TaqI		Tru9		ApaI	
	F	f	B	b	T	t	R	r	A	a
Case	116	26	54	62	90	52	112	30	85	57
%	81.7	18.3	45.76	52.54	63.4	36.6	78.8	21.2	59.8	40.2
Control	121	43	72	92	108	56	132	62	88	76
%	73.8	26.2	43.90	56.09	65.8	34.2	68.04	31.96	53.6	46.3
P-value*	0.099		0.661		0.498		0.028		0.275	

Table 4. Linkage disequilibrium between VDR gene polymorphism in control group*

	Tru 9	Taq I	Bsm I	Apa I
Fok I	0.900	0.629	0.868	0.724
Apa I	0.201	0.000	0.000	
Bsm I	0.039	0.000		
Taq I	0.201			

*All values are P-value for LD between polymorphism. FokI located at the 5' end and BsmI, ApaI, TaqI and Tru9 polymorphisms are located at the 3' end of the VDR gene. LD were significant between BsmI and Tru9, ApaI, TaqI and also between ApaI and TaqI polymorphisms.

The FokI VDR polymorphism, located in 5' end, is a T to C substitution in the first codon, abolishing the first translation initiation sites and results in a peptide lacking three amino acids, which increase the transcriptional activity of VDR. The resulting difference in VDR length by three amino acids may affect the function of the protein. However, we did not see any evidence for association between FokI polymorphism and the risk of thyroid cancer susceptibility. But a decreasing pattern in Odds ratio from FF to ff was seen (1.00, 0.87 and 0.37 for FF, Ff and ff respectively). This indicates a possible protective effect for ff genotype resulting from altered protein function.

The difference of allelic frequency of Tru9 between cases and controls ($p=0.028$) and lack of LD between Tru9 and other polymorphisms at the 3' end, suggests a possible association between this polymorphism and thyroid cancer risk.

This study seems to be one of the earliest in its kind. While no association between VDR polymorphisms and thyroid cancer was detected, the small sample size and subsequent low power of statistical tests have limited value of results.

We can not exclude the possibility that different polymorphisms in the VDR gene are associated with thyroid cancer. However it seems likely that other genomic and nongenomic factors will be more important in determining common thyroid cancer risk, a further larger study is needed to verify the present data.

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