RESEARCH COMMUNICATION

Lack of Association of VDR Polymorphisms with Thai Prostate Cancer as Compared with Benign Prostate Hyperplasia and Controls

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Abstract

While still relatively low as compared to rates in the Western world, prostate cancer is on the increase in Asia, presumably due to change in dietary and other lifestyle factors. One risk factor is reported to be vitamin D (VD) and therefore the function of its receptor (VDR) could be of importance. In the present study polymorphims with functional significance in the Bsm, Apa 1 and Taq 1 genes were therefore compared in 28 prostate cancer (CaP), 44 benign prostate hyperplasia (BPH) and 30 control cases in Thailand. None demonstrated any significant variation in distribution within these three groups and therefore we conclude that vitamin D may not be major risk factor for prostate cancer in this population. However, there is considerable variation in the distribution frequencies from country to country and this, combined with differences in sun exposure, means that the results may not be extrapolated to the general case.

Key Words: VDR polymorphisms - prostate cancer - benign prostate hyperplasia

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Introduction

While prostate cancer rates in Asia are still very much lower than in the Western world, they appear to be increasing in most countries and registries and particularly in Singapore Chinese and Malays, and in Japanese in Miyagi, presumably reflecting shifts in diet and other lifestyle factors (Sim et al., 2005) (see Table 1). In Thailand, the incidence overall is around 7 per 100,000, but there is considerable geographical variation and prevalence is much lower in Khon Kaen and Chiang mai than in the capital, Bangkok (Parkin et al., 2002).

High levels of testosterone and low levels of sex-hormone binding globulin (SHBG) are considered to be positively linked to prostate cancer development (Gann et al., 1996; Pienta and Esper, 1993). The calcium-vitamin D axis appears important (Chan et al., 2001; Giovannucci, 2005) and calcitriol, the active metabolite of vitamin D3, has several physiological roles. Therefore considerable attention has been focused on polymorphisms in the gene for the vitamin D receptor. There are four of note, Taq I, Apa I, Bsm I and Fok I, named after endonucleases, which have functional consequences (Zmuda et al., 2000). Since there appears to be considerable variation in the distribution of some of the polymorphisms (Bid et al., 2005), the present study was carried out to assess whether the Thai population might vary form others in Asia and also to assess any differences between controls and patients with prostate cancer or benign prostate hyperplasia.

Materials and Methods

Thai patients in Ramathibodi hospital were enrolled in

Table 1. Average Incidence Rates (/100,000) for Prostate Cancers in Selected Countries of Asia

	Iran[1] (1995-7)	India[2] (1993-6)	Pakistan[3] (1995-7)	China[1] (1995)	Mongolia[1] (1999)	Korea[4] (1993-7)	Japan[1] (1995)	Philippines[5] (1993-7)	Thailand[6] (1995-7)	Viet Nam[7] (1993-7)
Prostate	2.0	6.8	5.3	2.1	4.5*	5.4	10.0	22.3	6.8	3.8

Data from [1], Parkin and Vatanasapt, 2002; [2-7], Delhi, South Karachi,Seoul, Manila, Bangkok, Ho Chi Minh City, Parkin et al., 2002
* Prostate and testis combined

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the study and divided into three groups. Controls were outpatients who had no clinical symptoms of lower urinary tract disease and with a PSA level lower than 4 ng/ml. The BPH group who had histologically proven from TURP or TRUS biopsy and had PSA range //// to /////. The third CaP group was histopathologically proven to have prostatic cancer s, all adenocarcinomas. All subjects gave informed consent to the study and were examined for VDR polymorphisms with peripheral blood samples. Genomic DNA was isolated using a standard phenol chloroform method.

Reaction mixtures of 25 ml for used for the PCR (Bsm I, Apa I and Taq I polymorphisms) and amplification was achieved with a GeneAmp PCR system 2400 (Perkin Elmer) thermal cycler. Gels were visualised under UV and photographed for image analysis. The primers used were as follows:

Bsm I: forward, 5' AAC CAG CGG GAA GAG GTC AAG GG 3'; reverse, 5' CAA CCA AGA CTA CAA GTA CCG CGT CAG TGA 3'

Apa I: forward, 5' GCA ACT CCT CAT GGC TGA GGT CTC 3'; reverse, 5' CAG AGC ATG GAC AGG GAG CAA 3'

Taq I: forward, 5' GCA ACT CCT CAT GGC TGA GGT CTC 3'; reverse, 5' CAG AGC ATG GAC AGG GAG CAA 3'

The PCR conditions were as follows for all three: Initial denaturation at 95˚C for 3 min, followed by 30 cycles at 95˚C for 30 secs, 63˚C for 30 secs, 72˚C for 30 secs and final extension at 72˚C for 7 min.

Genotype frequencies of the VDR gene polymorphisms were determined according to the Hardy-Weinberg equilibrium. The Chi-square test and Mann Whitney-U test were performed to compare the allelic frequencies of the different groups using the computer software SPSS for Windows (version 10.0).

Table 2. Distributions of Cases and Controls for the Bsm 1, Apa 1 and Taq 1 Polymoprhisms

Group	Genotype (%)			Total
Bsm I	BB	Bb	bb	
Control	3 (10.0)	4 (13.3)	23 (76.7)	30 (100)
BPH	4 (9.1)	5 (11.4)	35 (79.5)	44 (100)
CAP	3 (10.7)	13 (14.3)	79 (75.0)	28 (100)
Total	10 (9.8)	13 (12.7)	79 (77.5)	102 (100)
Apa I	AA	Aa	aa	
Control	5 (16.7)	14 (46.7)	11 (36.7)	30 (100)
BPH	6 (13.6)	17 (38.6)	21 (47.7)	44 (100)
CAP	5 (17.9)	9 (32.1)	14 (50.0)	28 (100)
Total	16 (15.7)	40 (39.2)	46 (45.1)	102 (100)
Tsq I	TT	Tt	tt	
Control	21 (70.0)	8 (27.0)	1 (3.0)	30 (100)
BPH	38 (86.4)	6 (13.6)	0 (0.0)	44 (100)
CAP	22 (78.6)	6 (21.4)	0 (0.0)	28 (100)
Total	81 (81)	20 (20.6)	1 (1.0)	102 (100)

Results

The results for the genotype distributions are listed in Table 2. No significant differences were evident among the groups for any of the three polymorphisms.

Discussion

The present study revealed no significant variation in the allele frequencies for any of the three VDR polymorphisms studied with prostate disease state, while showing similar data overall to those earlier reported for a Thai population (Ongphiphadhanakul et al 1997) (see Table 3).

A large number of studies have been conducted to look at effects of the Fok I, BsmI, ApaI and TaqI polymorphisms of the VDR gene on risk of prostate cancer development. Overall results have been equivocal. While studies in Caucasians in the US have generally pointed to no influence (Ma et al., 1998; Blazer et al., 2000; Bousema et al., 2000; Cheteri et al., 2004; Oakley-Girvan et al., 2004; Hayes et al., 2005), in African Americans prostate cancer risk was associated with homozygosity for the F allele at the FokI site (Oakley-Girvan et al., 2004). Furthermore, race-adjusted combined analysis suggested that men who are homozygous for the t allele of TaqI (shown to correlate with higher serum levels of the active form of vitamin D) have one-third the risk of developing prostate cancer requiring prostatectomy compared to men who are heterozygotes or homozygous for the T allele (Taylor et al., 1996).

No significant association between VDR gene polymorphisms (BsmI, ApaI and TaqI) with familial prostate cancer risk in a Japanese population (Suzuki et al., 2003) or

Table 3. Racial Variation in the Distributions of the Apa I and Taq I Polymorphisms

Country	Genotype (%)			Reference
Apa I	AA	Aa	aa	
Thailand	16	40	45	Present study
Thailand	11	50	39	Ongphiphadhanakul et al (1997)
Japan	9	48	43	Tokita et al (1996)
China	10	36	54	Kung et al (1998)
Korea	3	28	69	Park et al (1999)
India South	38	46	16	Selvaraj et al (2003)
India North	36	44	20	Bid et al (2005)
Australia	26	51	23	Tokita et al (1996)
Greece	36	43	21	Fountas et al (1999)
Taq I	TT	Tt	tt	
Thailand	79	20	1	Present study
Thailand	83	17	0	Ongphiphadhanakul et al (1997)
Japan	77	22	1	Tokita et al (1996)
China	90	10	0	Kung et al (1998)
India North	49	40	11	Bid et al (2005)
Australia	36	48	16	Tokita et al (1996)
Greece	38	41	21	Fountas et al (1999)

in Brazilians (Maistro et al., 2004). However, the VDR TaqI polymorphism was found to be associated with a group of men in Saudi Arabia with BPH who are at an increase risk of CaP, providing a potential tool to assist prediction strategies (Tayeb et al 2004). Geographic localization could clearly be important in the definition of the genetic risk profile and a three-fold increase in prostate cancer risk was linked with the less active vitamin D receptor allele (the T allele from VDR TaqI polymorphism) in southern Europe(Medeiros et al., 2002). Furthermore, evidence from Taiwan also indicated the VDR BsmI polymorphism to play a significant role in the development of prostate cancer (Huang et al., 2004).

A meta-analysis showed the four polymorphisms are unlikely to be major determinants of susceptibility to prostate cancer on a wide population basis (Ntais et al., 2003). However, VDR variants may influence prostate cancer risk dependent on the extent of UVR exposure (Bodiwala et al., 2004). Geographic patterns in the US lend support to the hypothesis that UV radiation may protect against clinical prostate cancer (Hanchette and Schwartz, 1992). Furthermore, reduced risk of advanced prostate cancer was associated with high sun exposure and significant risk reductions with the high-activity alleles FokI FF or Ff, TaqI tt, and BglI BB genotypes in the presence of high sun exposure (John et al., 2005). For the vitamin D receptor gene, positive associations have in fact been found between prostate volume as a surrogate for hyperplasia and the Taq 1 polymorphism and the AA genotype of the Bsm 1 polymorphism (Mullan et al., 2006).

As shown in Table 3, there are great variations in the distributions of VDR polymorphisms across the races, with Caucasians and Aryan populations in India, for example, having higher proportions of the AA genotype of Apa I and the tt genotype of Taq I, relative to Eastern Asians. This is indicative of some evolutionary pressure and functional consequences, presumably related to skin colour and exposure to UV. The problem with resaerch limited to a country like Thailand is that the entire population is exposed to a great deal of sunlight. Thus future comparisons should perhaps be made by collaboration of research groups in different parts of the world, with the focus on variation not only in the genotype but also the major related environmental factors.

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