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Cross sectional studies on vitamin D receptor gene TaqI polymorphisms in healthy individuals from south Indian population

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Abstract
Vitamin D interacts with its receptor to play an important role in calcium homeostasis by regulating bone cell growth and differentiation, intestinal calcium absorption and parathyroid hormone secretion. The gene encoding VDR is located on Chromosome 12cen-q12, it has multiple gene polymorphisms in exon 2 and 3'UTR region (SNPs). TaqI (rs731236) are adjacent restriction fragment length polymorphisms (RFLP) which is located in the region of intron 8/exon 9. The following study aimed to investigate the distribution of VDR TaqI in unrelated healthy individuals in south Indian. Mouth wash were collected from 108 unrelated normal individuals (Male 54 and Female 54) from Kodaikanal, Tamilnadu. The investigation was done in accordance with the ethical principles outlined by the Indian Council of Medical Research (ICMR) guidelines for medical research involving human subjects and informed consent was obtained from volunteers. Genotyping Taq I, 2000bp restriction site polymorphism was done using PCR-RFLP with specific primers to amplify the fragment from the isolated DNA as per. Genotype frequencies were calculated in EXCEL and Hardy Weinberg equilibrium was tested using chi square with VASSARSTAT. Out of the 108 subject analyzed for TaqI (rs 731236), the following genotypic frequencies were obtained: TT 21%, Tt 63%, tt 17%. The south Indian TaqI (rs 731236) allele and genotype frequencies are statistically significant and hence genetically different from those Asian population Chinese-Hong Kong, Thailand, Japanese-Okinawa and North Indian-lucknow, Sahariya tribe, Bhil tribe, Chatttisgrah tribe, North Central Muslims, North Central, South East, North Indian-USA. In this study no significant difference in the distribution of genotypes and alleles of TaqI (rs 731236) were found between South Indian individuals and the Caucasian American, Caucasian UK, Caucasian Portugal, White North Carolina (Medeiros 2002), Black Pennsylvania, Australia, North Carolina, White Minnesota, Austria Caucasian-France, Caucasian-Sweden, Greece. Thus these populations were similar to south Indian Populations. Allelic and genotypic heterogeneity at the VDR TaqI locus between different ethnic populations may lead to differences in the pathogenesis of diseases.

Keywords: vitamin D receptor, TaqI, PCR-RFLP, osteoporosis

1. Introduction
Vitamin D receptor endocrine system is involved in a variety of biological processes including bone metabolism, modulation of the immune response. Vitamin D interacts with its receptor to play an important role in calcium homeostasis by regulating bone cell growth and differentiation, intestinal calcium absorption and parathyroid hormone secretion [1]. The gene encoding VDR is located on Chromosome 12cen-q12, it has multiple gene polymorphisms in exon 2 and 3'UTR region (SNPs).These are VDR-FokI (rs2228570), VDR-BsmI (rs1544410), VDR-TaqI (rs731236) and VDR-ApaI (rs7975232). TaqI (rs731236) are adjacent restriction fragment length polymorphisms (RFLP) which is located in the region of intron 8/exon 9 depending on the presence or absence of a Taq1 restriction site in each allele, products are digested into two fragments of 1700 and 300 bp (T allele: absence of the restriction site) or three fragments of 2000, 1700 and 300 bp (t allele: presence of the restriction site). Individuals are generally classified as TT, Tt or tt. The TT genotype has been shown to be associated with lower circulating levels of active vitamin D3 [2]. The vitamin D receptor gene (VDR), has been commonly associated with several diseases [3]. Gene – environment interactions are probably involved in most complex disease [4]. The action of vitamin D depends on the functional status of the VDR, which has polymorphic variants it is originally claimed that the polymorphic variation at the VDR locus may account for up to 75% of the genetic contribution to bone mass [5].

This gene encodes the nuclear hormone receptor for vitamin D3. This receptor also functions as a receptor for the secondary bile acid lithocholic acid. The receptor belongs to the family of trans-acting transcriptional regulatory factors and shows sequence similarity to the steroid and thyroid hormone receptors. Downstream targets of this nuclear hormone receptor are principally involved in mineral metabolism though the receptor regulates a variety of other metabolic pathways, such as those involved in the immune response and cancer. Mutations in this gene are associated with type II vitamin D-resistant rickets. A single nucleotide polymorphism in the initiation codon results in an alternate translation start site three codons downstream. Alternative splicing results in multiple transcript variants encoding different proteins [6].

Epidemiological and laboratory investigations have convincingly shown that vitamin D deficiency is associated with several common diseases, including Osteoporosis rickets

and other bone diseases, diabetes, cardiovascular diseases, autoimmune diseases, tuberculosis and cancer. Severe vitamin D deficiency is the cause of nutritional rickets that existed as a true endemic disease in many Western countries for several centuries and was also endemic in "Negro" children in North America and some children in wealthy families in India, all related to low endogenous vitamin D synthesis by lack of exposure to sunlight and low dietary vitamin D intake. Although such type of rickets can be easily prevented or cured by vitamin D supplementation, the disease is still endemic in several areas of the world, especially in the Middle East and Gulf States, Northern India and Northern China and Mongolia [7, 8]. In these children with vitamin D deficiency, the serum concentrations of 25-hydroxyvitamin D (25OHD) are usually well below 10 (and mostly below 5) ng /ml, in line with the threshold for 25OHD needed for active intestinal calcium absorption [9].

1,25-Dihydroxyvitamin D3 (1,25(OH) 2D3, calcitriol), the biologically most active naturally occurring metabolite of vitamin D, has been shown to regulate the growth and differentiation of various cell types, including cancer cells. Furthermore, there is recent evidence of regulatory effects on cell death, tumour invasion and angiogenesis in cancer. In agreement with these findings, the importance of the vitamin D endocrine system for cancer is now increasingly being recognized [10].

The following study aims to investigate the distribution of VDR Taq I in unrelated healthy individuals in south Indian.

2. Materials and Methods

2.1 Subjects

Mouth wash were collected from 108 unrelated normal individuals (Male 54 and Female 54) from Kodaikanal, Tamilnadu. It was ensured the volunteers participating in this study were all informed of the purpose and outcome of the study. For genotyping purpose random samples one per household were selected. The investigation was done in accordance with the ethical principles outlined by the Indian Council of Medical Research (ICMR) guidelines for medical research involving human subjects and informed consent was obtained from volunteers. All volunteers were above the age of 18 years. DNA was extracted from the mouth wash collected using a modified protocol of Ausubel [11].

2.2 Genotyping

Genotyping TaqI, 2000bp restriction site polymorphism was done using PCR-RFLP with specific primers to amplify the fragment from the isolated DNA as per Flugge., [12]. Taq I primer F 5′-CAA CCA AGA CTA CAA GTA CCG CGT CAG TGA-3′ R 5′-CAC TTC GAG CAC AAG GGG CGT TAG C-3′ Initial denaturation was at 95^0C for 2 minutes. 30 cycles of denaturation at 96^0C for 30 seconds which was followed by primer annealing at 56^0C for 60 seconds and by extension at 72^0C for 135 seconds. Final extension of 72^0C for 5 minutes was given. For Restriction digestion of the PCR Products the reaction mix was prepared as follows: 10X buffer 2X, Restriction enzyme 1U for a total volume of 20µl of the PCR product and incubated at 65°C for 2 hour and the product were analyzed by electrophoresis on 1.5% of agarose gel (Figure:1).

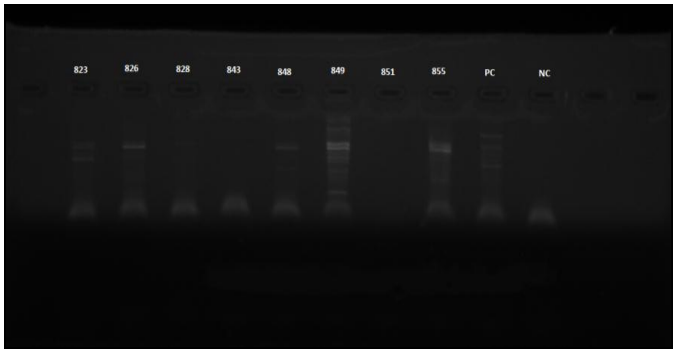

Fig 1: Gel Image of VDR

2.3 Statistical Analysis

Chi square test was done to compare the allele and genotype frequencies of South Indian healthy individuals to different population using the 2*3 contingency table and 2*2 contingency table vassarstats online calculator [13].

3. Results

The distribution of VDR Taq I (rs 731236) genotype and allele frequencies in the South Indian population are shown in Table 1.Out of the 24 subject analyzed for Taq I (rs 731236), the following genotypic frequencies were obtained: TT 21%, Tt 63%, tt 17% and the allele frequencies were obtained: T 54%, t 46% (Table.1)

Table 1: Genotype and Allele Frequency Distribution of Population

Ethinicity	N	Genotype % Frequency			P value	Allele % Frequency		P value	Reference
Chinese(Hong Kong)	144	90	10	0	***	95	5	***	Kung 1996
Thailand	84	83	17	0	***	92	8	***	Ongphiphadhanakul 1997 [14]
Japanese(Okinawa)	1431	79	20	2	***	88	12	***	Morita 2004 [15]
Caucasian American	41	37	44	20	NS	59	41	NS	Kibel 1998 [16]
Caucasian European(UK)	154	37	44	19	NS	59	41	NS	Luscombe 2001 [17]
Caucasian European (Portugal)	211	36	45	19	NS	58	42	NS	Medeiros l 2002 [18]
Black Pennsylvania	101	32	53	15	NS	58	42	NS	Zmuda 1997 [19]
Australia	518	36	48	16	NS	60	40	NS	Tokita 1996 [20]
Caucasian(France)	189	33	49	18	NS	57	43	NS	Garnero 2005 [21]
Mexican California	101	51	40	9	**	71	29	NS	Gross 1996 [22]
White North Carolina	162	33	45	22	NS	55	45	NS	Taylor 1996 [23]
White Minnesota	130	41	44	15	NS	63	37	NS	Riggs 1995 [24]
Austria	163	12	49	39	NS	36	64	NS	Ewald 1996 [25]

Caucasian (Sweden)	100	34	54	12	NS	61	39	NS	Carling 1997 [26]
Greece	53	38	41	21	NS	59	41	NS	Fountas 1999 [27]
Brazilians	192	49	36	15	*	67	33	NS	Gentil 2009 [28]
Indiana(Indianapolis)	250	15	48	37	NS	39	61	NS	Hustmyer 1994 [29]
North Indian-Lucknow	346	49	40	11	**	66	34	**	Bid *et al*. 2005 [30]
Sahariya Tribe	377	54	41	5	**	283	94	**	Sharma 2011 [31]
Bhil Tribe	95	47	48	5	*	67	28	*	Sharma 2011 [31]
Chhattisgarh Tribe	93	51	49	0	***	70	23	***	Sharma 2011 [31]
North Central Muslims	217	43	50	7	*	69	148	*	Sharma 2011 [31]
North Central	1021	45	45	10	*	689	332	*	Sharma 2011 [31]
South East	646	58	36	6	***	491	155	***	Sharma 2011 [31]
North Indian-USA	143	49	43	8	**	101	42	**	Bhanushali 2009 [32]
South Indian	108	21	63	17		54	46		Current Study

P value*=<0.05, P value**=<0.01, P value***=<0.001, NS=not significant

In the frequency distribution VDR gene Taq I polymorphism T allele showed highest frequency in Asian population Chinese-Hong Kong (95%), Thailand (92%). Lowest frequency was showed in North Central Muslims (32%) and Austria (36%).t allele showed highest frequency in North Central Muslims (68%) and Austria (64%).lowest frequency was showed in Chinese-Hong Kong (5%) and Thailand (8%) (Figure.2)

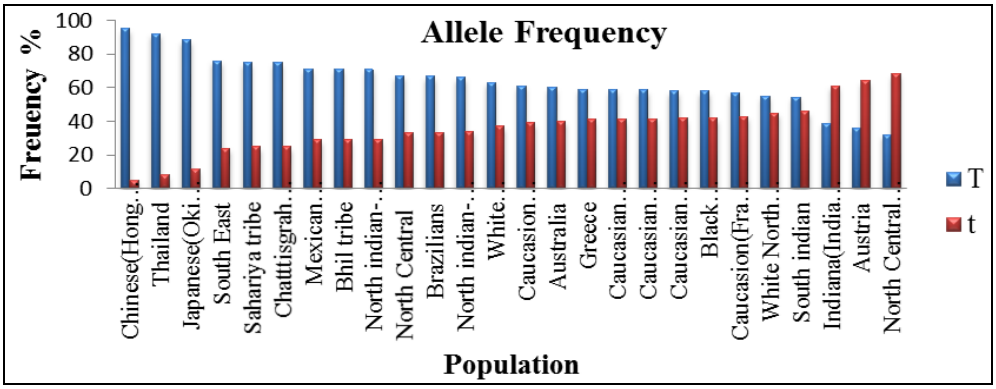

Fig 2: Allele Frequency Distribution of VDR TaqI polymorphisms in Compiled Population

Genotype frequency of VDR TaqI polymorphisms in complied population TT genotype showed highest frequency in Chinese-Hong Kong (90%), Thailand (83%) and Japanese-Okinawa (79%).lowest frequency in Austria (12%), Indiana-Indianapolis (15%) and South Indian (21%).

tt genotype showed highest frequency in Austria (39%) andIndiana-Indianapolis (37%). Lowest frequency was seen in Japanese-Okinawa (2%), Bhil tribe (5%), Sahariya tribe (5%), South East (6%), North Central Muslims (7%), North Indian-USA (8%), Mexican California (9%), North Central (10%).Chinese-Hong Kong, Thailand and Chhattisgarh tribe populations did not have tt genotype.

Tt heterozygote genotype frequency showed highest frequency in South Indian (63%), Caucasian-Sweden (54%) and Black Pennsylvania (53%).Lowest frequency was seen in Chinese-Hong Kong (10%), Thailand (17%) and Japanese-Okinawa (20%) (Figure.3).

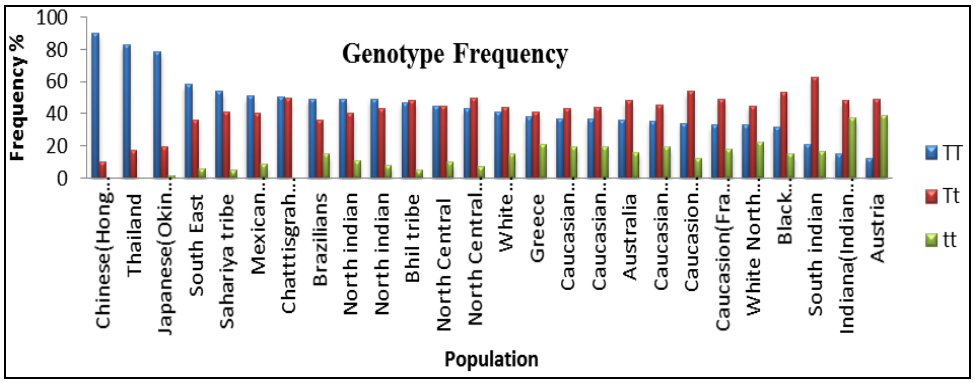

Fig 3: Genotype Frequency Distribution of VDR TaqI polymorphisms in Compiled Population

In this study no significant difference in the distribution of genotypes and alleles of TaqI (rs 731236) were found between South Indian individuals and the Caucasian American, Caucasian UK, Caucasian Portugal, White North Carolina (Medeiros 2002) [18], Black Pennsylvania, Australia, North Carolina, White Minnesota, Austria Caucasian-France, Caucasian-Sweden, Greece (Bid 2005) [30]. While allelic frequency was statistically significant in Brazilians (Gentil 2009) [28] and Mexican California (Gross 1996) [22], indicating that genotype distribution was significantly different in these two populations.

The south Indian TaqI (rs 731236) allele and genotype frequencies are statistically significant from those Asian population Chinese-Hong Kong (Kung 1996), Thailand (Ongphiphadhanakul 1997) [14], Japanese-Okinawa (Morita 2004) [15] and Indian population North Indian-lucknow, (Bid 2005) [30], Sahariya tribe, Bhil tribe, Chatttisgrah tribe, North Central Muslims, North Central, South East (Sharma 2011), North Indian-USA (Bhanushali 2009) [31, 32].

4. Discussion

The investigation of genetic variation can reveal the crucial determinant of gene- environmental interactions which is leads to various complex disease such as osteoporosis, hyper parathyroidism, Grave's disease, Type I-diabetes mellitus. Single nucleotide polymorphisms are highly scattered throughout the genome and high degree of variability make these informative genetic markers useful for disease susceptibility. This could have future implications for preventive and early intervention strategies.

Bhanushalli *et al*. [32] reported VDR gene polymorphisms have been associated with multiple traits and disease phenotypes like primary hyperparathyroidism, Grave's disease, Type I-diabetes mellitus and osteoporosis. Vitamin D function mediates its effects via the VDR which is a potent regulator of bone and calcium homeostasis as well as in immune modulation, cellular differentiation and replication in different target tissues [31].

Vitamin D receptor gene is mediated by Vitamin D which is responsible regulator of bone metabolism- absorption of calcium and desorption calcium as well as immune modulation, cellular differentiation and replication in different target tissues. The possible explanation for association of the Taq1 genotype with Vitamin D levels could be that polymorphisms in the VDR gene are known to influence calcium metabolism, which in turn plays an important role in feedback mechanism of Vitamin D levels. Alternatively it is also possible that the Taq I polymorphism may be in linkage disequilibrium with another marker that may be the true causative factor influencing the Vitamin D levels [9, 5] .

Indian population is believed to be most diverse because of different socio-cultural traditions. A number of possible reasons could explain the discrepancy of the results collected in different populations. First, there is allelic heterogeneity between different ethnic populations, which is exemplified by the extreme low frequency of tt genotype in South Indian as compared to Chinese Hong-Kong. The variation in our Indian population from the rest of the world population signifies the impact of ethnicity. Thus this kind of study may form the basis for future establishment of epidemiological and clinical databases. Allelic association studies are in progress with several chronic inflammatory and degenerative diseases in which VDR may be involved. In the long run, these studies may help in determining disease susceptibility and clinical management [29, 5].The genetic effect of VDR may be modulated by differences in environmental factors that affect BMD. A specific genotype may result in a phenotype only in certain environments, and result in a different phenotype in a different environment [4].

5. Conclusion

The present investigation of frequency of VDR TaqI polymorphisms not only vary between our population and Caucasians, but also vary from other Asian countries like Chinese-Hong Kong (TT-90%, Tt-10%, tt-0), Thailand (TT-83%,Tt-17%,tt-0%) and Japanese-Okinawa(TT-79%, Tt-20%, tt-2%). The frequency of the TaqI genotypes in the present study also shows different results than that of a study conducted in North Indian populations. A number of possible reasons could explain the discrepancy of the results collected in different populations. First, there is allelic heterogeneity between different ethnic populations, which is exemplified by the extreme low frequency of genotype in South Indian as compared to Chinese-Hong Kong.

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