Vitamin D Receptor Gene Polymorphism and Growth Pattern in Egyptian Rachitic Children

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Abstract: Nutritional rickets may be caused by either calcium or vitamin D deficiency. Vitamin D affects skeletal metabolism indirectly. The association between Vitamin D receptor (VDR) gene polymorphisms and genetic and environmental factors plays a role in the majority of cases. Several studies reported association between rickets and VDR gene polymorphism and growth parameters. Vitamin D affects skeletal metabolism by regulating calcium and phosphate homeostasis. The aim of this study was to examine the association between VDR gene polymorphism and vitamin D deficiency in Egyptian children with rickets and assess the relationship between the VDR gene polymorphisms and growth parameters**.** The study included 42 (16 girls and 26 boys) patients recruited from AL-Zharaa hospital, AL-Azhar University. Their age ranged from 4-36 months. Forty eight healthy individuals matched in age and sex with patients was recruited for comparison.VDR gene *ApaI*, *FokI*, and *TaqI* polymorphisms, biochemical and growth parameters were studied. Results showed that the most common VDR genotype was *Ff* among patients and *Aa* among controls, with no significant differences. The allele frequency showed significant increase in the "f" (*FokI*) allele in patients compared to controls (33.3% vs 20.8%, P=0.04). While, there were no significant differences between patients and controls in frequency of *TaqI* and *ApaI* alleles. The frequencies of combinations of VDR genotypes for the *FokI*, *ApaI*, and *TaqI* polymorphic sites, were significantly different between rachitic and control subjects ($p < 0.01$). The AaFfTT genotype was the most frequent one among the rachitic group, while the AaFFTT is the most predominant in the control group. The FF and tt genotypes were associated with reduced SDS of weight and height. This denotes that the VDR polymorphism has functional significance on growth parameters. In conclusions, the study shows that there is a relation between VDR gene polymorphisms and susceptibility to rickets. These results might help in risk assessment of rickets and in predicting response to treatment.

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1. Introduction:

Nutritional rickets is gaining the attention of public health professionals and clinicians world wide as the disease remains an endemic problem in many developing countries and has re-emerged in a number of developed countries, where it was thought that the disease has been almost eradicated (Thacher *et al*., 2006). In Egypt, vitamin D deficiency rickets continues to be a public health problem despite abundant sun shine all the year (Pettifor, 2008). Limited sun shine exposure due to more time indoors to watch TV and work on computer or avoiding sun shine internationally for fear of air pollution and skin cancer development have been reported as causes (Chesney, 2001; Hatun *et al*., 2007). Vitamin D regulates calcium and phosphate homeostasis in the body and has a positive impact of bone mineralization (Holick, 2006). The active form of vitamin D (1, 25-dihydroxyvitamin D3) exerts its effect on the target tissues through the VDR. Many tissues contain the VDR and thus the active form of vitamin D is expected to affect these tissues and cells like epidermis, macrophages, prostate, pancreas, parathyroid gland ((Fischer *et al*., 2000; Bora *et al*., 2008; Valdivielso and Fernadez, 2006; Bikle, 2007).

The emerging field in nutrition science, so called nutritional genomics (nutrigenomics) draws attentions to the fact that certain conditions of diseases may be linked to polymorphisms that individual carry. Presence of certain polymorphisms renders the most susceptible for certain disease even in the presence of recommended intake of offending nutrients whether is so for vitamin D and calcium is not clear, there have been some studies conducted in Africa indicating a possible risk between VDR polymorphisms and rickets (Fischer *et al*., 2000; Bora *et al*., 2008).

Vitamin D has direct effects on the skeleton, and the active metabolites regulate differentiation,

proliferation, and migration of osteoblasts and of chondrocytes of the epiphyseal growth plate, cells determining skeletal growth.VDR polymorphism could rather be related to growth and parameters of body constitution (Suarez *et al*., 1997; Minamitani *et al*., 1998; Lorentzon *et al*., 2000).

The vitamin D3 receptor (VDR) is an intracellular hormone receptor, which specifically binds to the active form of vitamin D (1, 25-dihydroxyvitamin D3 or calcitriol). It interacts with target-cell nuclei and produces a variety of biologic effects. The VDR protein is encoded by the VDR gene, which is linked to 12q13.1. VDR gene is about 100 kb, consists of 9 exons and has highly polymorphic sites. Several polymorphisms in the VDR gene have been reported so far, including FokI, TaqI, and ApaI. FokI, which is a translation start codon polymorphism, is located in exon 2, and due to the T to C transition. The other polymorphism, which is localized in exon 9, is TaqI and ATT codon is converted to ATC, but either of them encodes isoleucine amino acid. ApaI is an intronic polymorphism, which is G/T transition, localized in intron 8 (Audi *et al*., 1999; Uitterlinden *et al*., 2004). The aim of this study was to examine the association between VDR gene polymorphism and vitamin D deficiency in Egyptian children with rickets and assess the relationship between the VDR gene polymorphism and growth parameters**.**

2. Patients and Methods:

This study included 42 rachitic children, 26 boys and 16 girls and 48 non rachitic control group, 23 boys and 25girls matched in sex and age. They were selected from those attending the out patients clinic of AL-Zahraa hospital, AL-Azhar University. Their age was ranged from 4-36 months. A formal consent letter from the parents of each child was obtained after explaining to them the whole procedure. The study was approved by the Ethics Committee of the Hospital. The studied groups; cases and control were subjected to full history taking with special emphasis on the type and quantity of milk fed during infancy, calcium and vitamin D supplements, sun exposure (2 hours of sunlight per week). Clinical examination was performed with special stress on clinical signs of rickets; cranoitabes, rachitic rosary, Harrison groove, delayed closure of fontanels, muscular hypotonia, spinal deformity, pigeon chest or bowed legs. Children with chronic renal, hepatic, malabsorption disorders, congenital bone deformities, hypophosphatemia were excluded. Anthropometric measurements were taken including: weight, height or length, head circumference, waist and hip circumference, and mid arm circumference. The anthropometric measurements and instruments used followed the International Biological Programmer (IBP) (Tanner *et al*., 1969). Measurements were taken on the left side of the body. Body mass index (BMI in kg/m2); and relative head circumference (head circumference / height) were calculated. Physical growth was assessed for each child by determining the standard deviation scores of weight, height, BMI, head and mid-upper arm circumference, using the Egyptian growth reference data (Ghalli *et al*., 2002). We calculated standard deviation score (SDS) independent of sex and age (child measurement minus population mean/population SD). Radiological assessment of rickets in all cases for wrist and ankle was done. Serum calcium, phosphate and alkaline phosphates levels were measured by standard methods. Peripheral venous blood samples were collected on EDTA. Genomic DNA was extracted from peripheral white blood cells using salting out procedure (Miller *et al*., 1988). DNA was amplified by polymerase chain reaction (PCR) and examined (by specific restriction enzymes) using the restriction fragment length polymorphism (RFLP) technique. The VDR genotype of each subject was identified according to the digestion pattern and alleles according to the presence (f, t, and a) or the absence (F, T, and A) of the FokI, TaqI, and ApaI, restriction enzyme cleavage sites, respectively. Each VDR markers were amplified as following:

FokI polymorphism

Patients and control subjects DNA was amplified by PCR reaction in 25 µl total volume for FokI containing 10 mM tris HCl, 200 µM dNTPs, 20 pmol from the primer sequences F: 5'-AGC TGG CCC TGG CAC TGACTC GCT CT-3' and R: 5'- ATG GAA ACA CCT TGC TTC TTC TCC CTC-3', 1.5 mM $MgCl_2$, 0.5u taq polymerase (fenzyme), and using 50-100 ng of DNA as template. The temperature sittings were as follows; five min at 94ºC, followed by 35 cycles of 95ºC for 60 sec, 68ºC for 60 sec and 72 ºC for 2 min followed by 72 ºC for 7 min as a final extension step.

The PCR product was electrophoresed on 2 % agarose gel stained with ethidium bromide. PCR product 265 bp were visualized on UV transilluminator with using molecular weight marker to determine the quality of PCR products. Then conduct 10 µl of PCR product to 1 unite restriction enzyme (FastDigest Fok-I, Fermentas) at 37 ºC for 15 min, followed by 65 ºC for 3 min for digestion. After digestion were loaded the products on 2% agarose gel stained with ethidium bromide to identify the digestion pattern. The FF genotype, homozygote of common allele its meaning absence of restriction site and showed one band at 265 bp. The ff genotype (homozygote of infrequent allele) generated two fragments at 196 bp and 69 bp. Presence of three

fragments at 265 bp, 196 bp and 69 bp was appearance as Ff.

TaqI polymorphism

The PCR cycle conditions were initially denaturized at 94 ºC for 4 min, followed by 35 cycles at 94 ºC for 60 sec, 68 ºC for 60 sec and 72 ºC for 2 min followed by 72 ºC for 7 min as a final extension step. In total volume 25 µl were containing 10 mM tris HCl, 200 µM dNTPs, 20 pmol from the primer sequences F;5'- CAG AGC ATG GAC AGG GAG CAA-3'and R: 5'-CAC TTC GAG CAC AAG GGG CGT TAG C-3', 1.5 mM $MgCl_2$, 0.5u taq polymerase (fenzyme), and using 50-100 ng of DNA as template. The PCR product was electrophoresed on 2 % agarose gel stained with ethidium bromide. PCR product 600 bp were visualized on UV transilluminator with using molecular weight marker to determine the quality of PCR product.

The PCR product was digested with the restriction enzyme Taq-I (fastDigest-Taq-I, fermentas) 10 unit for 15 min at 37 ºC and followed by 65 ºC for 3 min according to manufacturer's instructions. The digested samples were added to loading dye and size fractionated by electrophoresis in a 1.5 % agarose gel. Visualization after ethidium bromide staining was accomplished by UV transilluminator. Taq-I digestion revealed one obligatory restriction site, the homozygous TT (absence of the specific Taq-I restriction site) yielded bands of 500 bp and 210 bp. The homozygous tt exhibited 210 bp and the heterozygous Tt 290 bp fragments.

ApaI polymorphism

The PCR cycle conditions were initially denaturized at 94 ºC for 4 min, followed by 35 cycles at 94 ºC for 30 sec, 65 ºC for 30 sec, and 72 ºC for 2 min and final extension at 72 ºC for 4 min. In total volume 25 µl were containing 10 mM tris HCl, 200 µM dNTPs, 20 pmol from the primer sequences F:5'-CAA CCA AGA CTA CAA GTA CCG CGT CAG TGA-3' and R: 5'- CAC TTC GAG CAC AAG GGG CGT TAG C-3', 1.5 mM $MgCl_2$, 0.5u taq polymerase (Fenzyme), and using 50-100 ng of DNA as template. The PCR product was electrophoresed on 1.5% agarose gel stained with ethidium bromide to check the quality of reaction. The amplified 2000 bp PCR product was subjected to Apa-I restriction enzyme (FastDigest, Fermentas) for digestion. 10 µl of PCR product was digested with 10 units of Apa-I restriction enzyme in a 20 µl total volume using green buffer at 37 ºC for 15 min and followed by 65 ºC for 3 min . The Apa-I enzyme digested product was loaded on a 1.5% agarose gel stained with ethidium bromide. The 2000 bp was digested as a common allele A (wild type) and presence of restriction site resulting in 1700 bp and 300 bp was assigned as infrequent allele (mutant allele). Genotypes were exhibited as homozygote's for common allele AA and homozygotes for mutant allele aa. Presence of 2000 bp, 1700 bp and 300 bp fragments was exhibited as heterozygotes Aa.

Statistical Analysis

Statistical presentation and analysis of the results were carried out using SPSS software version 11. Statistical tests used included chi-square test, student's t test, analysis of variance, and tukey tests. Correlations were tested between VDR gene polymorphisms, growth pattern and biochemical markers of vitamin D deficiency rickets.

3. Results:

The present study was performed on 42 patients with rickets and 48 healthy individuals, with the mean age 11.9±3.3 months and 15.3±3.3, respectively. DNA was obtained from both groups for identification of their VDR genotypes and allelic frequency. The Hardy- Weinberg equilibrium was satisfied to verify the allelic frequency. Table 1 and Fig.1 show the distribution of each allele in patients and controls. It was *F* (66.7 %), *f* (33.3%), *T* (59.1%)**,** *t* (40.9%)**,** A (61.3%) and *a* (38.8%) in patients and it was *F* (79.2 %), f (20.8%), *T* (59.3%), *t* (40.7%)**,** A (59.7%) and *a* (40.3%) in controls. The most common VDR genotypes were *Ff* (47.6%), *Tt* (45.5%), and *Aa* (60%) among patients and *FF* (52.08%), *Tt* (41.8%), and *Aa* (67.7%) among controls, with no statistical significant differences. The allele frequency showed significant increase in the "*f*" (FokI) allele in patients compared to controls (33.3% vs 20.8%, $P < 0.04$). While, there were no significant differences in frequencies of TaqI and ApaI alleles between patients and controls.

Table 2 shows the frequency distribution of combinations of VDR genotypes for the *Fok* I, *Apa* I, and *Taq* I polymorphic sites in patients and controls. Statistical analysis shows significant difference between the rachitic and control subjects ($p < 0.015$). In the rachitic patients, the AaFfTT genotype is the most frequent (23.3%), followed by the AAFFTt genotype (13.3%). Regarding the control group AaFFTT is the most predominant (40%). Tables 3 and 4 show the associations of VDR *Apa* I, and *Taq* I and *Fok*I genotypes with biochemical parameters, in patients and controls, respectively. Table 3 shows that patients with the *FF* genotype had significant decrease in serum phosphate compared to patients with the *ff* genotype ($P<0.05$). Also, patients with *tt* genotype had significant decrease in serum phosphate compared to patients with *TT* genotype ($P<0.05$). There were no significant differences between VDR genotypes in control group (Table 4).

Table 5 shows the mean SDS of growth parameters in rachitic children by vitamin D receptor (VDR) genotypes. The values of SDS for the weight, height, BMI, as well as mid-upper arm circumference (MUAC) lied at the lower limits of reference Egyptian growth data for all VDR genotypes with no statistical significant differences. However, the delay of weight SDS and height SDS is more pronounced in FF genotype compared to other genotypes in the rachitic children. Also, the SDS of weight and SDS of height in patients with tt genotype were delayed, with statistical significant difference when compared to other genotypes in the rachitic children (Fig.2&3). Head circumference measurements show normal SDS values.

The amplified products of the VDR gene are shown in Fig. 4. Panel A shows Fok-I digestion. Absence of Fok-I restriction site 265 bp was assigned as the common allele F and the genotype was considered as homozygous FF as in lane (2&4). The presence of Fok-I restriction site 196 bp and 69 bp was assigned as mutant allele f and the genotype was homozygous ff as in lane 1. Presence of 265, 196 and 69 bp indicated that genotype is heterozygous Ff as in lane 3.

Panel B shows Apa-I digestion of the amplified products of the VDR gene. Absence of Apa-I restriction site 2000 bp was assigned as the common allele A and the genotype was considered as homozygous AA as in lane (2). The presence of Apa-I restriction site 1700 bp and 300 bp was assigned as mutant allele a and the genotype was homozygous aa as in lane 1. Presence of 2000, 1700 and 300 bp indicated that genotype is heterozygous Aa as I lane 3(data not shown).

Panel C shows Taq-I digestion of the amplified products of the VDR gene. Absence of Taq-I restriction site 520 bp was assigned as the common allele T and the genotype was considered as homozygous TT as in lane 3. The presence of Taq-I restriction site 320 bp and 200 bp was assigned as mutant allele t and the genotype was homozygous tt as in lane 2. Presence of 520, 320 and 200 bp indicated that genotype is heterozygous Tt as in lane1.

Table 1. VDR genotype Distribution & Allelic frequency in patients and control

Group	***VDR Genotypes (%)***			***P value***	***Allele frequency (%)***		***P value***
Rachitic children	*FF*	*Ff*	*ff*	**0.16**	***F***	***f***	**0.04**
	42.8%	**47.6%**	**9.5%**		**66.7%**	**33.3%**	
Normal children	**52.1%**	**22.9%**	**6.2%**		**79.2%**	**20.8%**	
Rachitic children	***TT***	***Tt***	***tt***	**0.93**	***T***	***t***	**0.55**
	36.4%	**45.5%**	**18.2%**		**59.1%**	**40.9%**	
Normal children	**37.2%**	**41.8%**	**20.9%**		**9.3%**	**40.7%**	
Rachitic children	***AA***	***Aa***	***aa***	**0.76**	***A***	***a***	**0.49**
	30%	**60%**	**10%**		**61.3%**	**38.8%**	
Normal children	**25.8%**	**67.7%**	**6.5%**		**59.7%**	**40.3%**	

Table 2. Distribution of VDR genotypes among patients and control

VDR genotype	Cases (%)	Control (%)	Total (%)	P value
Homozygous AAFFtt	3.3%		2%	
aaffTT	3.3%		2%	
aaFFTT		5%	2%	
AAffTT	3.3%		2%	
Heterozygous aaFFTt	6.6%	5%	6%	
AaffTT		5%	2%	.015
AaFftt		15%	6%	
AaffTT		5%	2%	
AaFfTt	13.3%		8%	
AaFfTT	23.3%		14%	
AaFFtt	10%		6%	
AaFFTt	10%	20%	14%	
AaFFTT	6.6%	40%	20%	
AAffTt	3.3%		2%	
AAFfTt	3.3%		2%	
AAFFTt	13.3%	10%	12%	

Table 3. Biochemical parameters of children with rickets by VDR genotypes

VDR genotype		Calcium (mg/dl) Mean±SD	Phosphate (mg/dl) Mean±SD	Alk.Ph (IU/liter) Mean±SD
ApaI	aa	8.6 ± 0.49	3.57 ± 1.24	730.25 ± 63.69
	Aa	7.72 ± 1.12	3.66 ± 0.96	627.0 ± 33.85
	AA	8.29 ±1.17	4.03 ± 1.11	499.0 ± 243.18
FofI	ff	8.17 ± 1.05	4.17 ± 1.19	574.5 ± 219.72
	Ff	7.95 ± 1.12	4.16 ± 1.26	518.85 ± 355.53
	FF	8.10 ± 1.29	3.34 ± 0.67*	653.77 ± 346.95
TaqI	tt	7.65 ± 2.03	2.83 ± 0.18*	284.35 ± 116.08
	Tt	8.07 ± 1.01	3.86 ± 1.20	587.06 ± 382.57
	TT	8.08 ± 0.95	4.19 ± 1.13	468.08 ± 296.84

* P < .05

Table 4. Biochemical parameters of normal children by VDR genotypes

VDR genotype		Calcium (mg/dl) Mean±SD	Phosphate (mg/dl) Mean±SD	Alk.Ph (IU/liter) Mean±SD
ApaI	aa	8.6 ± 0.56	5.0 ± 0.56	131. 0 ±12.72
	Aa	8.92 ± 0.83	4.8 ± 0.94	210.75 ± 45.45
	AA	9.13 ± 0.53	4.56 ± 0.90	195.37 ± 123.35
FokI	ff	9.0 ± 0. 29	4.00 ± 0. 46	272.0 ± 0. 54
	Ff	8.60 ± 0.38	4.87 ± 0.69	163.71 ± 59.03
	FF	9.26 ± 0.49	4.65 ± 0.82	194.00 ± 107.27
TaqI	tt	9.17 ± 1.09	3.80 ± 0.54	268.75 ± 154.57
	Tt	8.87 ± 0.29	5.01 ± 0.46	148.44 ± 35.29
	TT	9.12 ± 0.25	4.57 ± 0.67	180.75 ± 65.09

Table 5. The mean SDS of growth parameters in rachitic children by VDR genotypes

VDR genotype		*Weight SDS Mean ± SD*	*Height SDS Mean ± SD*	*BMI SDS Mean ± SD*	*HC SDS Mean ± SD*	*M UA SDS Mean ± SD*	*HC /Ht SDS Mean ± SD*
ApaI	aa	-1.54 ±1.29	-1.12±1.20	-1.31±0.76	0.62±1.8	-0.85±1.8	1.81±1.20
	Aa	-1.56±0.43	-1.11±0.97	-1.32±0.98	0.64±1.29	-0.96±0.58	1.83±1.37
	AA	-1.15±1.29	-1.12±1.20	-0.62±1.76	-0.52±1.8	-0.25±1.8	1.05±1.24
FokI	ff	-0.93±0.78	-0.51±0.75	-0.53±1.80	0.8±0.97	-0.52±0.99	1.31±0.87
	Ff	-0.94±0.73	-0.52±0.79	-0.67±1.02	0.7±1.27	-0.18±0.99	1.32±1.65
	FF	-1.92±0.78	-1.86±0.75*	-1.19±1.80	-0.6±0.97	-1.23±0.88	1.70±0.87
TaqI	tt	-1.96±0.01	-2.38±0.70*	-1.66±0.01	-0.22±0.01	-0.79±0.01	2.22±0.01
	Tt	-1.17±1.28	-1.24±1.17	-0.59±1.77	-0.05±1.93	-0.67±0.99	1.55±1.89
	TT	-1.02±0.28	-0.32±0.95	-0.37±0.34	0.68±0.15	-1.19±0.79	1.03±1.17

* P< .05

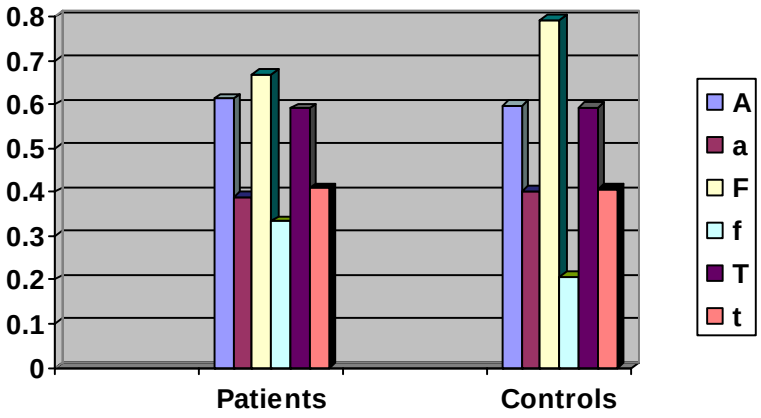

Fig.1. VDR Allelic Frequencies of Patients and Controls

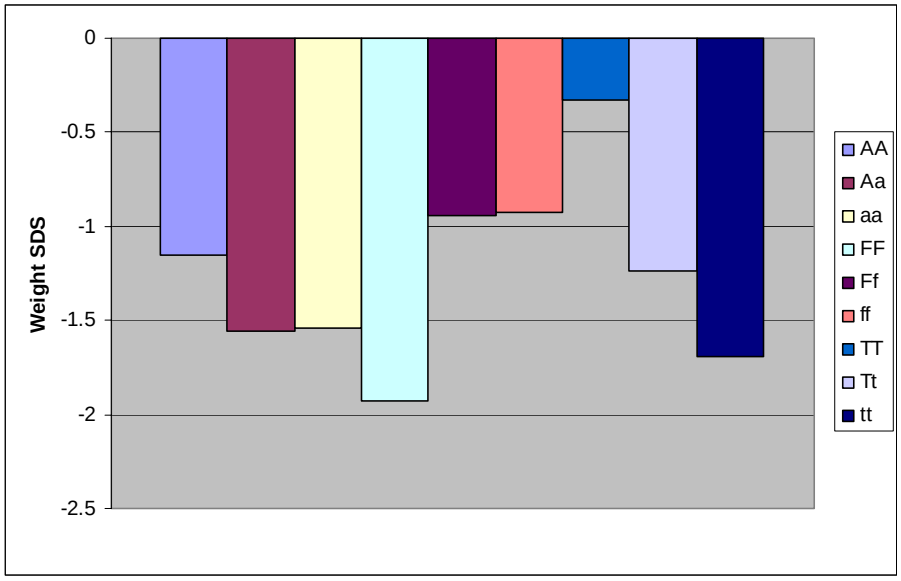

Fig.2. Mean SDS of weight in rachitic children by vitamin D receptor (VDR) genotypes

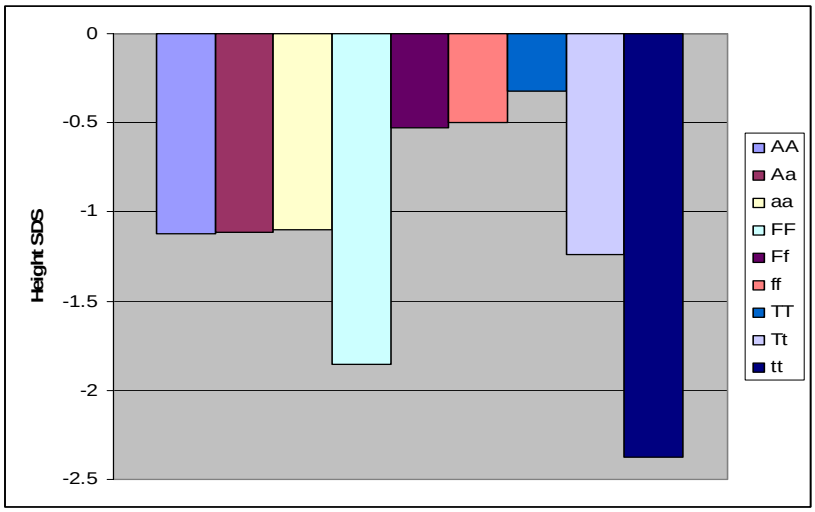

Fig.3. Mean SDS of height in rachitic children by VDR genotypes

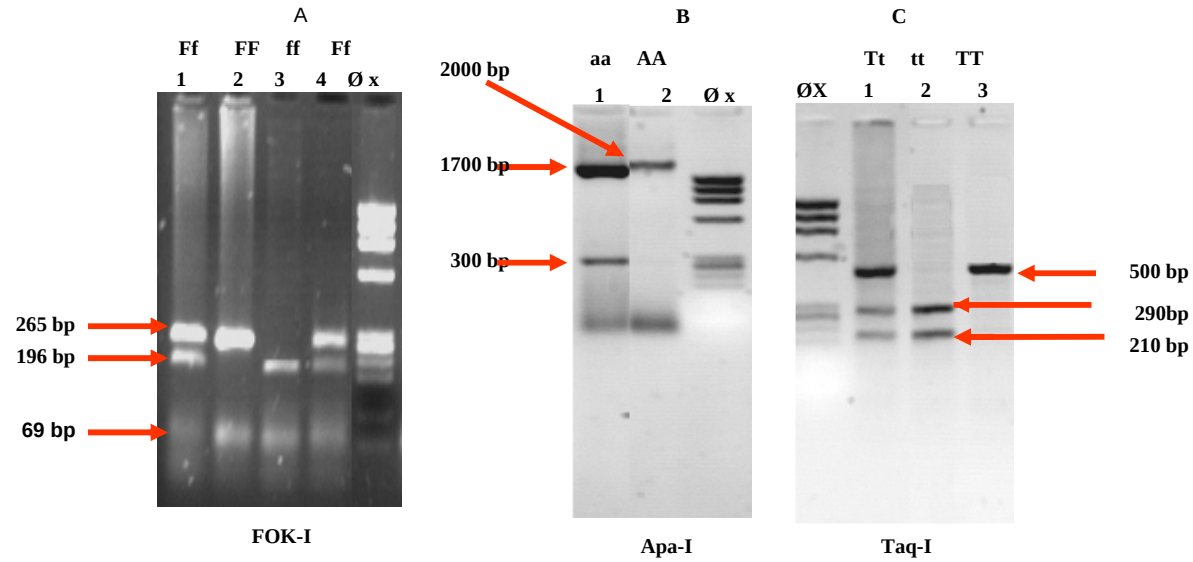

Fig. 4. PCR-RFLP analysis of the 3 VDR polymorphisms

Panel A Shows Fok-I digestion of the amplified products of the VDR gene. Absence of Fok-I restriction site 265 bp was assigned as the common allele F and the genotype was considered as homozygous FF as in lane (2&4). The presence of Fok-I restriction site 196 bp and 69 bp was assigned as mutant allele f and the genotype was homozygous ff as in lane 1. Presence of 265, 196 and 69 bp indicated that genotype is heterozygous Ff as in lane 3.
Panel B Shows Apa-I digestion of the amplified products of the VDR gene. Absence of Apa-I restriction site 2000 bp was assigned as the common allele A and the genotype was considered as homozygous AA as in lane (2). The presence of Apa-I restriction site 1700 bp and 300 bp was assigned as mutant allele a and the genotype was homozygous aa as in lane 1. Presence of2000, 1700 and 300 bp indicated that genotype is heterozygous Aa as I lane 3(data not shown).
Panel **C** Shows Taq-I digestion of the amplified products of the VDR gene. Absence of Taq-I restriction site 520 bp was assigned as the common allele **T** and the genotype was considered as homozygous **TT** as in lane 3. The presence of Taq-I restriction site 320 bp and 200 bp was assigned as mutant allele **t** and the genotype was homozygous **tt** as in lane 2. Presence of 520, 320 and 200 bp indicated that genotype is heterozygous **Tt** as in lane1.

4. Discussion:

Nutritional rickets may be caused by either vitamin D or calcium deficiency. Genetic and environmental factors play a role in the majority of cases (Baroncelli *et al*., 2008; Pettifor, 2008). The association between vitamin D receptor (VDR) polymorphisms and several diseases in different populations has been investigated (Park *et al*., 1999; Ozaki *et al*., 2000). The VDR gene polymorphism has been wildly used as a genetic marker for diseases related to calcium metabolism. Several polymorphisms in the VDR gene which are able to alter the activity of VDR proteins have been described (Filus *et al*., 2008). Expression and nuclear activation of the VDR are necessary for the effects of vitamin D. Several genetic variations have been identified in the VDR (Valdivielso and Fernandez; 2006). The VDR gene affects the activity of the receptor and subsequent downstream vitamin D mediated effects (Gao *et al*., 2010).

The most common genotype frequency in the present study in the Egyptian control group is *Aa* (67.7%) but this percentage is higher or nearly similar to other populations such as France 50 % (Garnero *et al*. (1995), Mexican, California 55%(Sainz *et al*., 1997), Indian 44% (Bid et al., 2005), black Pennsylvanian 46%(Zmuda et al., 1997) and Chinas 36%(Kung et al.; 1998). This may be due to the difference in the ethnic background of the population being studied. ApaI polymorphism is localized in 3' regulatory region and is in linkage disequilibrium with 3'UTR. It is an intronic polymorphism, affecting neither splicing site nor transcription factor binding site. In this study we demonstrated that the frequencies of "A" allele and AA genotype were increased and Aa genotype was decreased in rachitic children compared to controls. This is in agreement with the study done by Bora *et al*. (2008) in the East of Turkey on vitamin D deficient rickets. However, the study of Wei-Ping *et al*. (2005) on Chinese children reported that the distribution of ApaI polymorphism was balanced between rickets and controls. Likewise Kaneko *et al*. (2007) indicated that VDR polymorphisms among cases did not differ significantly from those of controls in Mongolia. Although these polymorphisms seem to be non-functional; they can be used as a marker to detect a functional allele due to the linkage disequilibrium. The 3'UTR region of the VDR gene is involved in the regulation of gene expression, so these polymorphisms may play an important role in mRNA stability. It may be normal to find the different allelic frequencies among patients, due to the different ethnic background of the patients in different countries (Bora *et al*., 2008).

FokI is an exonic polymorphism, which leads to T/C transition, and variant alleles generate two VDR gene products that differ in length by three amino acids (Ferrari et al., 1998; Gross et al., 1998). Many Studies have been performed to determine whether there is a difference in functionality between these receptor variants. Arai et al., (1997) concluded that the *F* allele (short form) functioned better than the *f* allele (long form) in transactivation assays using a transfected vitamin D responsive element (VDRE)-reporter gene construct. Also, Remus et al.,(1998)and Jurutka et al., (1998) reported that the *F* allele (producing a shorter VDR) has higher transactivation activity, possibly because of better ability to dimerize with retinoid X receptors and bind to transcription factor II B (TFIIB), a coactivator of vitamin D transactivation. In contrast, Gross et al., (1998) were unable to detect a difference in VDR affinity or abundance, messenger RNA (mRNA) stability, or transactivation activity between *ff* and *FF* cells.

In the present study, no significant difference was found in VDR genotypes (FokI) in patients against controls. This is in agreement with the study done by Baroncelli et al., (2008) on Turkish and Egyptian rachitic children. Lu et al., (2003) studied the VDR gene (FokI) in Chinese rachitic and control subjects and they found a significant difference in the frequency distribution of VDR genotypes (FokI) between the two groups and in *F* allele frequency. Our study showed significant increase in *f* allele frequency of FokI polymorphism in patients,however there was no significant difference in the frequency distribution of VDR genotypes (FokI)). The *F* allele confers a transcriptionally somewhat more efficient VDR (Arai et al.; 1997), and its decreased prevalence in patients suggests that it may increase predisposition to rickets in children. Fischer et al., (2000) studied the VDR genotypes in Nigerian Children and found that *"F"* allele was more abundant in rickets subjects. In Turkey, the frequency of the *F* allele was increased and that of the *f* allele was decreased in patients against controls (Baroncelli et al.; 2008). The frequency of the *Ff* genotype in the present control group (22.9%) is lower than other populations such as France 47 % (Correa et al., 1999), Mexican California 48 % (Gross et al., 1996), Indian 49% (Bid et al., 2005) and Japanese 51% (Minamitani et al., 1998).

This result may be explained by the small number of subjects in this study or it might be because of the differences in ethnic backgrounds.

The current study demonstrated no significant difference in allele and genotype frequencies of TaqI among rickets and controls. Likewise, Fischer et al., 2000) and Kaneko et al., (2007) reported that neither allele nor genotype frequencies of TaqI were

significantly different between rickets and controls in Nigeria and Mongolia populations, respectively. In contrast, Bora et al., (2008) found a significant increase in *TT* and *tt* genotypes and decrease in *Tt* genotypes in Turkish rachitic children. TaqI polymorphism is localized in 3' regulatory region and is in linkage disequilibrium with 3'UTR. TaqI is exonic polymorphism that does not affect the amino acid sequence of encoded protein. Although these polymorphisms seem to be non functional, they can be used as a marker to detect a functional allele due to the linkage disequilibrium. The 3'UTR region of the VDR gene is involved in the regulation of gene expression, so these polymorphisms may play an important role in mRNA stability. Frequencies of combinations of genotypes at different sites were not significantly different between rachitic and community subjects in Nigerian (Fischer et al; 2000). In contrast our findings showed that the frequencies of combinations of VDR genotypes for the Fok I, Apa I, and Taq I polymorphic sites in both groups, were significantly different between rachitic and control subjects ($p < 0.015$). Uitterlinden et al., (2004) reported that it is possible that different allelic frequencies and VDR genotypes among populations can occur due to the gene-gene and gene-environment interactions.

In the present study, the most common VDR genotypes were *Ff* among patients and *FF* among controls. These findings support the evidence that *FF* genotype is advantageous for good bone mineralization and the prevention of rickets (Arai et al.; 1997 and Remus et al.; 1998). Children with the *FF* genotype had increased intestinal calcium absorption and increased bone mineral density compared with *Ff* heterozygotes and *ff* homozygotes. In healthy adolescents greater calcium absorption was found in *FF* homozygotes, compared with those of *ff* homozygotes and *Ff* heterozygotes (Ames et al., 1999); however, the positive effect of the *FF* genotype is limited whether dietary calcium is severely restricted (Abrams et al., 2005). Baroncelli et al., (2008) suggested that it is the interaction of VDR polymorphism with reduced calcium intake and vitamin D status that could determine the individual susceptibility to developing rickets in Egyptian patients.

The polymorphisms in the VDR gene might cause mild defects in VDR function and cause rickets (Malloy et al., 1999). The data of Suarez et al., (1997) and Minamitani et al., (1998) indicated that VDR polymorphism could be related to parameters of body growth. In the present study, the values of SDS for the weight, height, BMI, and MUAC among the studied rachitic group showed values that lies at the extreme lower ends of the reference Egyptian growth data (Ghalli et al., 2002). This is in agreement with the study of Robinson et al., (2004) on rachitic Australian children, who reported that rickets has a negative impact on growth and the cases presenting with nutritional rickets had a lower weight SDS. Bora et al., (2008) recorded that, the Turkish vitamin D deficient rickets patients are at risk of growth retardation. Vitamin D is likely to regulate growth via effects on bone size (Lorentzon et al., 2000). It is critically important for the development, growth, and maintenance of a healthy skeleton throughout life (Holick, 2003). It affects skeletal metabolism indirectly via regulating calcium and phosphate homeostasis through stimulation of intestinal absorption of these ions (Bouillon et al., 1995).

The weight SDS and height SDS among VDR genotypes for polymorphisms in *ApaI* were nearly of the same values in our patients. As regards the FokI genotypes the delay is more pronounced in the rachitic children with *FF* genotype .These results explain that growth delay in rickets is more influenced by FokI. Studies on polymorphism in the *VDR* gene suggested a role in skeletal mineralization, with the restriction fragment length polymorphism *F/f*, as defined by the endonuclease FokI, conferring a greater transcriptional VDR activity for the *F* than the *f* allele (Arai et al., 1997; Thakkinstian et al.; 2004).

In the present study patients with the *FF* genotype had significant decrease in serum phosphorus, compared to patients with the *ff* genotype. Also, patients with the *FF* genotype showed pronounced delay in height as compared to the other genotypes with statistical significant difference ($P<0.05$). Likewise, Lu et al., (2003) reported that *FF* genotypes were more common in patients suffering from vitamin D deficient rickets. This denotes that the polymorphic variation at the FokI VDR locus has functional significance. It has also been observed that there is a relationship between growth and FokI VDR polymorphisms in a population (Minamitani et al, 1998; Tao et al., 1998).

Tao et al., 1998 reported that Girls with genotype *TT* were heavier and taller than those with *tt*. This is in agreement with our study on the weight SDS and height SDS among VDR genotypes for polymorphisms in *TaqI*. We found that the patients with rickets with *tt* genotype were shorter than patients with *TT* with a statistical significant difference. In England, Keen et al., (1997) reported a significant association between female infant weight and a *TaqI* polymorphism within the VDR gene. They concluded that FokI and TaqI have a determinant effect on bone mineral metabolism and growth in rickets and the frequency of VDR polymorphisms in the rachitic children may determine growth delay in rickets.

Mutations affecting genes implicated in vitamin D metabolism or vitamin D receptor (VDR) functions are responsible for severe alterations in skeletal growth. These polymorphisms appear to be associated unequivocally with biochemical variables of calcium and phosphate metabolism polymorphisms and might cause mild defects in VDR function (Lu et al.; 2003). We therefore postulated that VDR polymorphisms might predict susceptibility to develop rickets in Egyptian children

The findings of the present study indicate that the growth in rachitic children may be regulated by mechanisms that are mediated through vitamin D and its receptor. The results reinforce the suggestion that VDR polymorphisms may play an important role in parameters of phospho-calcium metabolism and growth in rickets. This might help in risk assessment of rickets and in predicting response to treatment. Moreover, if a relationship could be established between certain polymorphisms and vitamin D deficient rickets personalized dietetic approach of nutrigenomics, will be applied to carriers of these particular polymorphisms and might be supplemented with more than the recommended daily dose to prevent the development of rickets.

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5. References:

1. Abrams SA, Griffin IJ, Hawthorne KM, Chen Z, Gunn SK, Wilde M, Darlington G, Shypailo RJ, Ellis KJ. 2005 Vitamin D receptor Fok1 polymorphisms affect calcium absorption, kinetics, and bone mineralization rates during puberty. J Bone Miner Res; 20: 945–953.
2. Ames SK, Ellis KJ, Gunn SK, Copeland KC, Abrams SA. 1999 Vitamin D receptor gene Fok-I polymorphism predicts calcium absorption and bone mineral density in children. J Bone Miner Res; 14:740-746.
3. Arai H, Miyamoto K, Taketani Y, Yamamoto H, Iemori Y, Morita K, Tonai T, Nishisho T, Mori S, Takeda E. 1997 A vitamin D receptor gene polymorphism in the translation initiation codon: effect on protein activity and relation to bone mineral density in Japanese women. J Bone Miner Res; 12:915–921.
4. Audi L, Ramirez-Garcia M, Carrascosa A. 1999 Genetic determinants of bone mass. Horm Res; 51:105-123.
5. Baroncelli GI, Bereket A, El Kholy M, Audı L`, Cesur Y, Ozkan B, Rashad M, Ferna´ ndez-Cancio M, Weisman Y, Saggese G, and Hochberg Z 2008 Rickets in the Middle East: Role of Environment and Genetic Predisposition. J Clin Endocrinol Metab; 93:1743–1750.
6. Bid HK, Mishra DK, Mittal RD. 2005 Vitamin-D Receptor (VDR) Gene (Fok-I, Taq-I& Apa-I) Polymorphisms in Healthy Individuals from North Indian Population.Asian Pacific Journal of Cancer Prevention; 6:147-152.
7. Bikle DD. 2007 What is the new in vitamin D .Current Opin Rheumatol; 19:383-388.
8. Bora G, Ozkan B, Erden DD et al. 2008 Vitamin D receptor gene polymorphism in Turkish children with vitamin D deficient rickets .The Turkish Journal of Pediatrics; 50:30-33.
9. Bouillon R, Okamura WH, Norman AW. 1995 Structure- function relationships in the vitamin D endocrine system. Endocr Rev; 16:200 –257.
10. Chesney RW. 2001 Vitamin D deficiency rickets. Rev Endocr Metab Disord; 2:145-151.
11. Correa-Cerro L, Berthon P, Haussler J, et al. 1999 Vitamin D receptor polymorphisms as markers in prostate cancer. Hum Genet; 105:281-287.
12. Ferrari S, Rizzoli R, Manen D, Slosman D, Bonjour JP. 1998 Vitamin D Receptor gene start codon polymorphisms (*Fok*I) and bone mineral density: Interaction with age, dietary calcium and 39-end region polymorphisms. J Bone Miner Res; 13**:**925-930.
13. Filus A, Trzmiel A, Kuliczkowsk-Płaksej J, Tworowska U, Jedrzejuk D, Milewicz A, Medraś M. 2008 Relationship between vitamin D receptor BsmI and FokI polymorphisms and anthropometric and biochemical parameters describing metabolic syndrome. Aging Male. Sep; 11(3):134-9
14. Fischer PR, Thacher TD, Pettifor JM, Jorde LB, Eccleshall TR, Feldman D. 2000 Vitamin D receptor polymorphisms and nutritional rickets in Nigerian children. J Bone Miner Res; 15: 2206-2210.
15. Garnero P, Borel O, Sornay-Rendu E, et al. 1995 Vitamin D receptor gene polymorphisms do not predict bone turnover and bone mass in healthy premenopausal women. J Bone Miner Res; 10:1283-1288.
16. Gao L, Tao Y, Zhang L, Jin Q. 2010 Vitamin D receptor genetic polymorphisms and tuberculosis: updated systematic review and meta-analysis. Int J Tuberc Lung Dis; 14:15-23.
17. Ghalli I, Salah N, Hussien F, Erfan, M, El-Ruby M, Mazen I, Sabry M, Abd El-Razik, M, Saad M, Hossney L, Ismaail S. and Abd El-Dayem S, et al. In: Proceedings of the 1st National Congress for Egyptian Growth Curves, Cairo University, 11 December 2003, Cairo. Published in Cresceve

Nelmondo, 2008, Ferring Company. Eds: Sartorio, A., Buckler, J.M.H. and Marazzi, N. Egyptian Growth Curves 2002 for Infants, Children and Adolescents.
18. Gross C, Krishnan AV, Malloy PJ, Eccleshall TR, Zhao X-Y, Feldman D. 1998 The vitamin D receptor gene starts codon polymorphism: A functional analysis of FokI variants. J Bone Miner Res; 13:1691–1699.
19. Gross C, Eccleshall TR, Malloy PJ, et al., 1996 The presence of a polymorphism at the translation initiation site of the vitamin D receptor gene is associated with low bone mineral density in postmenopausal Mexican-American women. J Bone Miner Res;11:1850-1855.
20. Hatun S, Bereket A, Ozkan B et al 2007 Free vitamin D supplementation for every infant in Turkey. Dis Child; 92:373-374.
21. Holick MF. 2006 Resurrection of the vitamin D deficiency and rickets. J Clin Invest; 116:2062-2071.
22. Jurutka PW, Remus LS, Thompson PD, Whitfield GK, Hsieh JC, Zitzer H, Tavakkoli P, Galligan MA, Dang HTL, Haussler CA, Blanco JCG, Ozato K, Haussler MR. 1998 Molecular characterization of polymorphic human vitamin D receptors that are associated with differences in bone mineral density: Residues N-terminal of the first zinc finger enhance transcriptional activity by contacting TFIIB. Bone 23 (Suppl 5); S186. (abstract).
23. Kaneko A, Urnaa V, Nakamura K, et al. 2007 Vitamin D receptor polymorphism among rickets children in Mongolia. J Epidemiol; 17: 25-29.
24. Keen RW, Egger P, Fall C, Major PJ., Lanchbury JS., Spector TD, Cooper C. 1997 Polymorphisms of the Vitamin D Receptor, Infant Growth, and Adult Bone Mass. Calcif Tissue Int; 60:233–235.
25. Kung AW, Yeung SS, Lau KS. 1998 Vitamin D receptor gene polymorphisms and peak bone mass in southern Chinese women.Bone; 22,389-93.
26. Lorentzon M, Lorentzon R, Nordstrom P. 2000 Vitamin D receptor gene polymorphism is associated with birth height, growth to adolescence, and adult stature in healthy Caucasian men: a cross-sectional and longitudinal study. J Clin Endocrinol Metab; 85:1666–1670.
27. Lu HJ, Li HL, Hao P, Li JM, Zhou LF. 2003 Association of the vitamin D receptor gene start codon polymorphism with vitamin D deficiency rickets. Zhonghua Er Ke Zhi; 41: 493-496.
28. Malloy PJ, Pike JW, Feldman D. 1999 The vitamin D receptor and the syndrome of hereditary 1, 25-dihydroxyvitamin D resistant rickets. Endocr Rev; 20:156–188.
29. Miller SA, Dykes DD, and Polesky F. 1988 A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res**;** 16: 1215-1218.
30. Minamitani K, Takahashi Y, Minagawa M, Yasuda T, Niimi H. 1998 Difference in height associated with a translation start site polymorphism in the vitamin D receptor gene. Pediatr Res; 44: 628–632.
31. Ozaki Y, Nomura S, Nagahama M, Yoshimura C, Kagawa H, Fukuhara S. 2000 Vitamin D receptor genotype, and renal disorder in Japanese patients with systemic lupus erythematosus. Nephron; 85: 86-91.
32. Park BS, Park JS, Lee DY, Youn JI, Kim IG. 1999 Vitamin D receptor polymorphism is associated with psoriasis. J Investig Dermatol Symp Proc; 112: 113-116.
33. Pettifor JM. 2008 Vitamin D or calcium deficiency rickets in infants, children: A global prescriptive. Indian J Med Res; 127: 245-249.
34. Remus LS, Whitfield GK, Jurutka PW, Zitzer H, Oza AK,Dang HTL, Haussler CA, Galligan MA, Thatcher ML, Haussler MR. 1998 Functional evaluation of endogenous VDR alleles in human fibroblasts cell lines: Relative contribution of F/f and L/S genotypes to 1,25(OH)2D3-elicited VDR transactivation ability. Bone; 23:S186 (abstract).
35. Robinson PD, Ho¨ gler W, Craig ME, Verge CF, Walker JL, Piper AC, Woodhead HJ, Cowell CT, Ambler GR. 2006 The re-emerging burden of rickets: a decade of experience from Sydney. Arch Dis Child; 91:564–568.
36. Sainz J, Van Tornout JM, et al. 1997 Vitamin D-receptorgene polymorphisms and bone density in prepubertal American girls of Mexican descent. N Engl J Med, 337: 77-82.
37. Suarez F, Zeghoud F, Rossignol C, Walrant O, Garabedian M. 1997 Association between vitamin D receptor gene polymorphism and sex-dependent growth during the first two years of life. J Clin Endocrinol Metab; 82: 2966 –2970.
38. Tanner JM, Hiernaux J, Jerman S. Human Biology 1969. A guide to Field Methods. IBP Hand book. (ed) by Weiner J, lourie J.Blackwell Scientific Publications, Oxford and Edinburgh. U. K
39. Tao C, Yu T, Garnett S, Briody J, Knight J, Woodhead H, Cowel CT. 1998 Vitamin D receptor alleles predict growth and bone density in girls. Arch Dis Chil; 79:488-493.
40. Thacher TD, Fischer PR, Strand MA et al. 2006 Nutritional rickets around the world : causes and future directions . Ann Trop pediatric; 26:1-16.
41. Thakkinstian A, D'Este C, Eisman J, Nguyen T, Attia J. 2004 Meta-analysis of molecular

association studies: vitamin D receptor gene polymorphisms and BMD as a case study. J Bone Miner Res; 19: 419-428.
42. Uitterlinden AG, Fang Y, Meurs van JB, Leeuwen van H, Pols HA. 2004 Vitamin D receptor gene polymorphisms in relation to vitamin D related disease states. J Steroid Biochem Mol Biol; 89-90: 187-193.
43. Uitterlinden AG, Fang Y, Meurs van JB, Pols HA, Leeuwen van JP. 2004 Genetics and biology of vitamin D receptor polymorphisms. Gene; 338: 143-156.
44. Valdivielso MJ, Fernandez E. 2006 Vitamin D receptor polymorphisms and disease. Clini Chim Acta; 371:1-12.
45. Wei-Ping, Jian-ping Y, Lian-qing LI, et al. 2005 Association of vitamin D receptor gene ApaI polymorphism with vitamin D deficiency rickets. Chin J Pediatr; 43: 514-516.
46. Zmuda JM, Cauley JA, Danielson ME, Wolf RL, Ferrell RE. 1997 Vitamin D receptor gene polymorphisms, bone turnover and rates of bone loss in older African-American women. J Bone Miner Res; 12:1446–1452.

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