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Research Article
Vitamin D Receptor Gene Polymorphism among Egyptian Obese Children

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Abstract

Background: The prevalence of childhood obesity has increased during the last few years in many parts of the world. It has been suggested that obesity results from the interaction between genetic and environmental factors. Vitamin D receptor (VDR) gene polymorphism is associated with several conditions involving postnatal growth, insulin sensitivity and Body Mass Index (BMI). **Objective:** The aim of the present study was to assess the genetic contribution of VDR polymorphisms to the pathogenetic mechanism of obesity. **Methodology:** The study included 110 children (50 obese and 60 control), 6-16 years old, recruited from Al-Azhar University Hospital, Damietta; during the period from July, 2015 to April, 2016. For each child, three single nucleotide VDR gene polymorphisms were genotyped: *Fok*I, *Apa*I and *Taq*I using the Restriction Fragment Length Polymorphism (RFLP) technique. **Results:** Obese children had significantly low vitamin D (28.7±7.9 ng mL^{-1}) than control group (32.4±4.2 ng mL^{-1}, p = 0.002). Regarding genotype distributions, no significant difference between cases and controls was observed in genotype and allele frequencies of VDR-*Fok*I and VDR-*Apa*I. For the *Taq*I polymorphism, there were significant differences in genotype frequencies of VDR-*Taq*I between obesity and control groups (p = 0.039) (OR for 'tt' allele = 3.47, 95% CI: 1.1-10.7). The 't' allele distribution in the obesity group was significantly higher than control group (p = 0.003) and the OR for 't' was 2.33 (95% CI: 1.34-4.1). Among obesity-only children, homozygotes of the VDR-*Taq*I 'tt' variant were associated with increased triglyceride levels (p = 0.03) and HDL levels (p = 0.006). **Conclusion:** These results further support a role for VDR-*Taq*I polymorphism as risk factor for obesity and suggest its further validation inlarger independent populations as well as highlight a target for functional analysis towards therapeutic intervention in obese individuals.

Key words: Obesity, vitamin D, VDR gene, polymorphism, genetics, children, body mass index, lipid profile, blood pressure

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Obesity prevalence has increased during the past decades in children and adolescents, leading to a significant current and future health burden[1]. Childhood obesity has been known to be associated with a range of health problems, which may last until adult life and cause premature morbidity and mortality[2].

The pathogenesis of obesity is complex including genetic and environmental factors that are not yet fully clarified[3]. Gene variations account for as much as 40-80% of causes in obesity[4].

Vitamin D is a group of fat-soluble secosteroids, best known for its role in bone health by enhancing the intestinal absorption of calcium and phosphate[5]. Accumulating evidence also strongly suggests many important extra-skeletal effects[6], such as modification of immune competence, cancer risk, blood pressure regulation, infectious disease risk and propensity to autoimmune diseases[7].

Although, the mechanisms underlying vitamin D in obesity is still incompletely explained, poor vitamin D status has been associated with obesity in humans, but vitamin D supplementation failed to decrease body weight[8].

The action of this vitamin is mediated through vitamin D receptor (VDR), a nuclear transcription-regulating factor that signals the synthesis of proteins involved in bone mineral homeostasis and cell-cycle regulation, while also interacts with other cell-signaling pathways that influence the development of obesity[9]. The VDR gene, located at chromosome region 12q13, includes 9 exons and 8 introns and expressed in many tissues[10].

Frequent polymorphisms in the VDR gene were reported to be associated with a variety of physiological and pathological phenotypes in many populations. These phenotypes included variations in body weight[11], insulin sensitivity[12] and a susceptibility[13] to type 1 or type 2 diabetes[14].

One of the main evidence for a role of VDR in obesity was derived from transgenic mice studies that over-express human VDR in adipocytes which leads to a marked decrease in energy expenditure and induction of obesity[15].

Several studies investigated the association between VDR gene polymorphism and adulthood obesity[16-18] but there is a lack of studies that focused on the role of VDR gene polymorphism in the development of childhood-onset obesity.

In Egypt, it has been reported that obesity is prevalent among 20% of school children[19], which represents a public health problem. Thus, it is essential to study the factors associated with obesity, which will contribute critically to our basic knowledge of the disease etio-pathogenesis and the identification of new targets for therapeutic intervention; hence, the present study was designed to evaluate the role of vitamin D receptor gene polymorphism in Egyptian obese children.

MATERIALS AND METHODS

Subjects: The present case control study consisted of 50 obese children (BMI≥95 percentile) and 60 normal weight (BMI<85 percentile and ≥5 percentile) children, which were chosen as a control group; their ages ranged from 6-16 years. They were recruited from outpatient clinic at Al-Azhar University Hospital, Damietta; during the period from July, 2015 to April, 2016. Children with suspected endocrinal or genetic obesity, children with hepatic, renal, diabetes mellitus, hypertension, rickets and malabsorption disorders were excluded from the study. Children receiving medication that affect weight as anticonvulsants, steroids, vitamin D or multivitamins were also excluded from the study.

The study was approved by the local ethical committee. Written informed consent was obtained from parents.

Methods and computations: Weight was measured by spring scale sensitive to 100 g (Seca, type® 80). The child was weighed with minimal clothes, no shoes and determined to the nearest 0.1 kg. Height was estimated using a measuring stick which was fixed to a vertical flat surface such as a wall and approximated to the nearest 0.5 cm. The BMI was calculated by dividing the weight in kilogram by the square height in meters[20]. Obesity was determined using BMI percentiles matched for age and sex, 2-21 years using the Egyptian growth reference data[21]. Normal weight is defined as BMI from the 5-85th percentile for age and sex. Overweight is BMI from the 85-95th percentile for age and sex. Obesity is BMI≥95th percentile for age and sex[22]. Height, body weight and BMI were expressed as z-scores and/or percentiles to allow pooled comparisons of values from children of different ages and sex. Blood pressure was measured by using a 1042 Riester sphygmomanometer (Jungingen, Germany) of appropriate cuff sizes according to mid arm circumference. Three readings were taken 2 min apart and the average of the two last values was used in the analyses.

Sampling and assessment: A venous blood sample was withdrawn from each child after an overnight fast of 12 h. Serum was separated and stored at -20°C until the time of assay. Twenty five-hydroxy vitamin D was assayed using

quantitative enzyme immunoassay [Cobas E601 analyzer (Roche)], using the vitamin D3 (25-OH) assay as recommended by the manufacturer and the remaining of venous serum sample was assayed for serum fasting glucose, cholesterol and triglyceride. Serum glucose was measured by the glucose oxidase technique (Siemens ADVIA 1800). Serum cholesterol and triglyceride were measured by the homogeneus colorimetric enzyme technique (Roche, Cobas 8000).

DNA studies: Genomic DNA was extracted from peripheral white blood cells using salting out procedure[23]. The 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 *Fok*I, *Taq*I and *Apa*I, restriction enzyme cleavage sites, respectively. The VDR markers were amplified as follow:

Initial denaturation at 94°C for 5 min, 35 cycles of 94°C for 30 sec, annealing temperature (*Apa*I and *Taq*I at 64°C, *Fok*I at 60°C) for 30 sec, 72°C for 30 sec and final extension of 72°C for 5 min. The primers were obtained for *Fok*I (F): 5'-AGC TGG CCC TGG CAC TGACTC GCT CT-3' and (R): 5'-ATG GAA ACA CCT TGC TTC TTC TCC CTC-3', for *Taq*I (F): 5'-CAG AGC ATG GAC AGG GAG CAA-3'and (R): 5'-CAC TTC GAG CAC AAG GGG CGT TAG C-3' and for *Apa*I (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'. For *Apa*I and *Taq*I, a fragment of 740 bp was amplified. Digestion with *Apa*I revealed two fragments of 515 and 225 bp in a 1.5% agarose gel which meant the presence of restriction site can be written as 'aa'. Digestion with *Taq*I in a 2.5% agarose gel showed the fragments of 290, 245 and 205 bp in the presence of polymorphic site written as 'tt'; fragments of 495 and 245 bp written as TT. For *Fok*I polymorphism, a fragment of 265 bp was digested into two fragments 196 and 69 bp in presence of *Fok*I site which recognized asff.

Statistical analysis: Data were analyzed using the SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). Quantitative data were expressed as the Mean±Standard Deviation (SD). For comparisons of data, the student's t test was used to compare between two means. Distribution of VDR genotypes in two groups were examined using chi-square test. The Odds Ratio (OR) was calculated to estimate the size effect of different alleles in obesity. For all tests, significance was considered if $p<0.05$.

RESULTS

Demographic and anthropometric data of studied children are demonstrated in Table 1. Obese children had significant elevation of total cholesterol, LDL cholesterol, triglycerides and low HDL cholesterol ($p<0.001$). Also, obese children had significantly low vitamin D than control group ($p = 0.002$) as present in Table 2. Regarding genotype distributions, no significant difference between cases and controls was observed in genotype and allele frequencies of VDR-*Fok*I ($p = 0.63$ and 0.74) and VDR-*Apa*I ($p = 0.82$ and 0.64). For the *Taq*I polymorphism, there were significant differences in genotype frequencies of VDR-*Taq*I between obesity and control groups ($p = 0.039$) (OR for 'tt' allele = 3.47, 95% CI: 1.1-10.7). The 't' allele distribution in the obesity group was significantly higher than control group ($p = 0.003$) and the OR for 't' was 2.33 (95% CI: 1.34-4.1) as shown in Table 3. Additionally, in obesity-only children carriers for the common allele for VDR were compared their frequency against the rare allele for all parameters of obesity. Homozygotes of the VDR-*Taq*I 't' variant were associated with increased triglyceride levels ($p = 0.03$) and HDL levels ($p = 0.006$) compared with heterozygotes and homozygotes of the 'T' allele in obesity subjects (Table 4).

DISCUSSION

Despite growing recognition of the problem, the obesity epidemic continues and obesity rates are increasing around the world[24]. Therefore, it is widely hoped that the

Table 1: Demographic and anthropometric parameter of the studied groups

	Obese group (n = 50)	Control group (n = 60)	p-value
Age (years)	11.60±3.90	12.10±3.2	0.46
Gender (males/females)	27/23	28/32	0.44
Height (percentile)	63.40±12.3	59.80±11.5	0.11
Height (z-score)	0.420±0.87	0.340±0.73	0.60
Weight (z-score)	2.530±0.58	0.140±0.42	<0.001*
BMI (z-score)	2.320±0.45	0.260±0.56	<0.001*
Systolic BP (mm Hg)	117.4±10.3	111.9±8.70	0.003*
Diastolic BP (mm Hg)	81.20±7.90	78.70±5.70	0.06

*Significant

Table 2: Fasting blood glucose, lipid profile and vitamin D levels of studied groups

	Obese group (n = 50)	Control group (n = 60)	p-value
Fasting glucose (mg dL^{-1})	83.2±5.10	81.7±4.60	0.11
Total cholesterol (mg dL^{-1})	179.6±12.5	150.6±14.4	<0.001*
HDL cholesterol (mg dL^{-1})	48.2±5.30	54.2±5.10	<0.001*
LDL cholesterol (mg dL^{-1})	119.2±13.2	96.7±15.9	<0.001*
Triglyceride (mg dL^{-1})	129.2±14.8	107.2±16.3	<0.001*
25-Hydroxy vitamin D (ng mL^{-1})	28.7±7.9	32.4±4.20	0.002*

*Significant

Table 3: Distribution of vitamin D gene polymorphism in studied groups

	Obese group (n = 50)	Control group (n = 60)	p-value	OR (95% CI)
FokI				
FF	20 (40%)	28 (47%)		0.76 (0.36-1.6)
Ff	22 (44%)	21 (35%)	0.63	1.46 (0.67-3.1)
ff	8 (16%)	11 (18%)		0.85 (0.3-2.30)
f	38 (38%)	43 (36%)	0.74	1.097 (0.63-1.9)
F	62 (62%)	77 (64%)		
ApaI				
AA	16 (32%)	16 (27%)		1.30 (0.57-2.9)
Aa	27 (54%)	35 (58%)	0.82	0.84 (0.39-1.8)
aa	7 (14%)	9 (15%)		0.92 (0.32-2.7)
a	41 (41%)	53 (44%)	0.64	0.88 (0.51-1.5)
A	59 (59%)	67 (56%)		
TaqI				
TT	14 (28%)	27 (48%)		0.47 (0.21-1.06)
Tt	24 (48%)	28 (67%)	0.039*	1.05 (0.5-2.20)
tt	12 (24%)	5 (5%)		3.47 (1.1-10.7)*
t	48 (44%)	34 (35%)	0.003*	2.33 (1.34-4.1)*
T	52 (56%)	86 (65%)		

*Significant

Table 4: Subject's anthropometric and laboratory profile according to *TaqI* gene among obese children

	VDR (TT+Tt)	VDR (tt)	p-value
Weight (z-score)	2.47±0.52	2.68±0.36	0.19
BMI (z-score)	2.28±0.43	2.43±0.38	0.28
Fasting glucose (mg dL^{-1})	84.40±5.90	82.90±4.70	0.43
Total cholesterol (mg dL^{-1})	176.10±11.8	181.30±10.2	0.18
HDL cholesterol (mg dL^{-1})	52.20±6.20	46.60±4.60	0.006*
LDL cholesterol (mg dL^{-1})	117.20±12.5	119.80±10.2	0.28
Triglyceride (mg dL^{-1})	122.50±15.3	133.40±12.1	0.03*
25-Hydroxy vitamin D (ng mL^{-1})	28.30±7.80	28.90±6.70	0.81

*Significant

identification of the genetic factors underlying the heritable risk of obesity will contribute to our basic knowledge of the biology of energy balance and might even highlight molecules and pathways that can be targeted for human intervention[16].

The VDR genes have been suggested to be potential key players in the pathogenetic mechanism of obesity[25]. Different ethnic gene and allele variations are in different frequencies, studies have revealed that VDR polymorphisms across ethnics were correlated with different incidences of many diseases. Thus, it was necessary to investigate the possible association between known polymorphisms in VDR genes and the development of obesity in Egyptian children.

Obesity is usually correlated with the higher prevalence of hypovitaminosis or the lower circulating 25(OH) D level in both pediatric as well as adult populations[26]. Association between hypovitaminosis D and obesity has been also confirmed in the present study. Similarly, a recent Egyptian study reported that the prevalence of 25(OH) D deficiency was higher in the obese group (29.9 ng mL^{-1}) than in the control group (39.7 ng mL^{-1}) with significant difference[27]. The inverse relationship between obesity and serum 25(OH) D concentrations may have several explanations, including deposition of vitamin D in body fat compartments, reduced release of vitamin D into systemic circulation and low exposure to sun light[28]. Until now, data regarding the role of vitamin D in obesity are inconclusive[29].

The present study found a strong association between VDR-*TaqI* 't' allele and obesity. This VDR polymorphism on itself or acting as an association signal for a nearby mutation due to linkage disequilibrium, as it happens to be the case for a microsatellite repeat marker in VDR's 3 UTR[30] could affect VDR's mRNA stability leading to an alteration in its protein expression levels[16]. Interestingly, when human VDR was over-expressed in the adipocytes of transgenic mice, this resulted ina significant decrease in energy expenditure and induction of obesity[30].

In children, there are few studies which investigated the role of VDR polymorphism relation with obesity. Ferrarezi *et al.*[31] reported association between VDR-*BsmI* polymorphism and height in a cohort of obese children and adolescents; however, there was no significant association between VDR-*TaqI* and VDR-*ApaI*.

In a recent study from Kingdom of Saudi Arabia; their data indicated that polymorphisms affecting the vitamin D/VDR axis play a role in obesity that is associated with an ongoing degree of inflammation, possibly resulting from alterations of gut permeability and microbial translocation. The associations with obesity were positive for all studied polymorphisms including *BsmI*, *ApaI* and *TaqI*[18].

In contrast, a Polish study reported that there was no statistically significant differences were noted for weight, height and BMI depending on four VDR genotypes (*BsmI*, *FokI*, *ApaI* and *TaqI*) at all three analyzed loci[32].

The influence of VDR polymorphisms on somatic development is one of many genetic factors determining growth and development as well as the involution processes. Moreover, it depends on many environmental factors (geographical, social, economic and cultural). Therefore, the results obtained in various populations are different.

Several studies confirmed the same association in adult's studies. The association between vitamin D receptor gene polymorphism (*TaqI*) and obesity was confirmed recently in Chinese population[17]. These results consistent with other studies in which the *TaqI* polymorphism presented a significantly higher weight and BMI[33,34].

Moreover, homozygotes of the VDR-*TaqI* 't' allele were associated with increased triglyceride levels and decreased HDL levels, something that suggests an exacerbation of lipid

accumulation and triggering of atherosclerosis. Similar results are found also in a Greek study[16].

CONCLUSION AND LIMITATION

The present study suggests that *Taq*I of VDR polymorphisms is associated with obesity in a sample of Egyptian children. The genotype 'tt' and allele 't' of *Taq*I may be potential predictors related to obesity. These results could help the definition of VDR fingerprints that predict an increased risk of developing obesity and might contribute to the identification of novel therapeutic strategies for this metabolic condition.

Despite the significance of our findings, these results should be taken cautiously as our study had some limitations, being mainly the relatively small sample size of obesity as the case-control design that may have allowed some elusive misclassification of controls. Further replication of our findings in larger independent cohorts could overcome these limitations and provide sufficient power to test for gene-gene and gene-diet interactions in order to disentangle the molecular basis of obesity.

REFERENCES

1. Ng, M., T. Fleming, M. Robinson, B. Thomson and N. Graetz *et al*., 2014. Global, regional and national prevalence of overweight and obesity in children and adults during 1980-2013: A systematic analysis for the Global burden of disease study 2013. Lancet, 384: 766-781.
2. Kelishadi, R, S.D. de Ferranti, R. Majdzadeh, J.A. O'Dea, A.K. Gupta and K. Adeli, 2013. Childhood obesity: Today and tomorrow's health challenge. J. Obesity. 10.1155/2013/208392.
3. Dubois, L., K.O. Kyvik, M. Girard, F. Tatone-Tokuda and D. Perusse *et al*., 2012. Genetic and environmental contributions to weight, height and BMI from birth to 19 years of age: An international study of over 12,000 twin pairs. PLoS ONE, Vol. 7. 10.1371/journal.pone.0030153.
4. Lee, Y.S., 2013. Genetics of nonsyndromic obesity. Curr. Opin. Pediatr., 25: 666-673.
5. Holick, M.F., 2007. Vitamin D deficiency. N. Engl. J. Med., 357: 266-281.
6. Rosen, C.J., J.S. Adams, D.D. Bikle, D.M. Black and M.B. Demay *et al*., 2012. The nonskeletal effects of vitamin D: An Endocrine society scientific statement. Endocr. Rev., 33: 456-492.
7. Hossein-Nezhad, A. and M.F. Holick, 2013. Vitamin D for health: A global perspective. Mayo Clinic Proc., 88: 720-755.
8. Bouillon, R., G. Carmeliet, L. Lieben, M. Watanabe and A. Perino *et al*., 2014. Vitamin D and energy homeostasis-of mice and men. Nat. Rev. Endocrinol., 10: 79-87.
9. Awad, A.B., L. Alappat and M. Valerio, 2012. Vitamin D and metabolic syndrome risk factors: Evidence and mechanisms. Crit. Rev. Food Sci. Nutr., 52: 103-112.
10. Taymans, S.E., S. Pack, E. Pak, Z. Orban, J. Barsony, Z. Zhuang and C.A. Stratakis, 1999. The human Vitamin D Receptor gene (*VDR*) is localized to region 12cen-q12 by fluorescent *in situ* hybridization and radiation hybrid mapping: Genetic and physical *VDR* map. J. Bone Mineral Res., 14: 1163-1166.
11. Grundberg, E., H. Brandstrom, E.L. Ribom, O. Ljunggren, H. Mallmin and A. Kindmark, 2004. Genetic variation in the human vitamin D receptor is associated with muscle strength, fat mass and body weight in Swedish women. Eur. J. Endocrinol., 150: 323-328.
12. Jain, R., P.R. von Hurst, W. Stonehouse, D.R. Love, C.M. Higgins and J. Coad, 2012. Association of vitamin D receptor gene polymorphisms with insulin resistance and response to vitamin D. Metab. Clin. Exp., 61: 293-301.
13. McDermott, M.F., A. Ramachandran, B.W. Ogunkolade, E. Aganna and D. Curtis *et al*., 1997. Allelic variation in the vitamin D receptor influences susceptibility to IDDM in Indian Asians. Diabetologia, 40: 971-975.
14. Oh, J.Y. and E. Barrett-Connor, 2002. Association between vitamin D receptor polymorphism and type 2 diabetes or metabolic syndrome in community-dwelling older adults: The Rancho Bernardo study. Metabolism, 51: 356-359.
15. Wong, K.E., J. Kong, W. Zhang, F.L. Szeto and H. Ye *et al*., 2011. Targeted expression of human vitamin D receptor in adipocytes decreases energy expenditure and induces obesity in mice. J. Biol. Chem., 286: 33804-33810.
16. Vasilopoulos, Y., T. Sarafidou, K. Kotsa, M. Papadimitriou and Y. Goutzelas *et al*., 2013. VDR TaqI is associated with obesity in the Greek population. Gene, 512: 237-239.
17. Fan, H.R., L.Q. Lin, H. Ma, Y. Li and C.H. Sun, 2015. Association between vitamin D receptor gene polymorphism (TaqI) and obesity in Chinese population. J. Genet., 94: 473-478.
18. Al-Daghri, N.M., F.R. Guerini, O.S. Al-Attas, M.S. Alokail and K.M. Alkharfy *et al*., 2014. Vitamin D receptor gene polymorphisms are associated with obesity and inflammosome activity. PLoS ONE, Vol. 9. 10.1371/journal.pone.0102141.
19. Ezzat, A.M., M.F. EL Gendy, D.R. Soliman and H.S.A.A. Abou Ghazy, 2011. Body mass index as an assessment tool for overweight and obesity in school children in El-Qalubia governorate. J. Am. Sci., 7: 240-250.
20. Kuczmarski, R.J., C.L. Ogden, S.S. Guo, L.M. Grummer-Strawn and K.M. Flegal *et al*., 2002. 2002 CDC growth charts for the United States: Methods and development. Vital Health Stat., 11: 1-190.

21. Ghali, I., N. Salah, F. Hussien, M. Erfan and M. El-Ruby *et al*., 2008. Egyptian Growth Curves for Infants, Children and Adolescents. In: Crecere Nel Mondo, Satorio, A., J.M.H. Buckler and N. Marazzi (Eds.). Ferring Publisher, Italy.
22. CDC., 2011. CDC grand rounds: Childhood obesity in the United States. Morbidity Mortality Weekly Rep., 60: 42-46.
23. Miller, S.A., D.D. Dykes and H.F. Polesky, 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res., 16: 1215-1215.
24. Mitchell, N., V. Catenacci, H.R. Wyatt and J.O. Hill, 2011. Obesity: Overview of an epidemic. Psychiatr. Clin. North Am., 34: 717-732.
25. Perusse, L. and C. Bouchard, 2000. Gene-diet interactions in obesity. Am. J. Clin. Nutr., 72: 1285s-1290s.
26. Lagunova, Z., A.C. Porojnicu, R. Vieth, F.A. Lindberg, S. Hexeberg and J. Moan, 2011. Serum 25-hydroxyvitamin D is a predictor of serum 1,25-dihydroxyvitamin D in overweight and obese patients. J. Nutr., 141: 112-117.
27. Hassan, N.E., S.A. El-Masry, R.A. El Banna, M.M. Abu Shady and M. Al-Tohamy *et al*., 2014. 25-hydroxy vitamin D, adiponectin levels and cardiometabolic risk factors in a sample of obese children. Macedonian J. Med. Sci., 7: 562-566.
28. Wortsman, J., L.Y. Matsuoka, T.C. Chen, Z. Lu and M.F. Holick, 2000. Decreased bioavailability of vitamin D in obesity. Am. J. Clin. Nutr., 72: 690-693.
29. Ross, A.C., J.E. Manson, S.A. Abrams, J.F. Aloia and P.M. Brannon *et al*., 2011. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: What clinicians need to know. J. Clin. Endocrinol. Metab., 96: 53-58.
30. Ingles, S.A., R.W. Haile, B.E. Henderson, L.N. Kolonel and G. Nakaichi *et al*., 1997. Strength of linkage disequilibrium between two vitamin D receptor markers in five ethnic groups: Implications for association studies. Cancer Epidemiol. Biomarkers Prev., 6: 93-98.
31. Ferrarezi, D.A.F., N. Bellili-Munoz, C. Nicolau, N. Cheurfa and I.C. Guazzelli *et al*., 2012. Allelic variations in the vitamin D receptor gene, insulin secretion and parents' heights are independently associated with height in obese children and adolescents. Metabolism, 61: 1413-1421.
32. Jakubowska-Pietkiewicz, E., I. Klich, W. Fendler, W. Mlynarski and D. Chlebna-Sokol, 2013. Effect of Vitamin D Receptor gene (*VDR*) polymorphism on body height in children-own experience. Postepy Higieny Medycyny Doswiadczalnej, 67: 873-878.
33. Binh, T.Q., Y. Nakahori, V.T.T. Hien, N.C. Khan, N.T. Lam, L.B. Mai and S. Yamamoto, 2011. Correlations between genetic variance and adiposity measures and gene×gene interactions for obesity in postmenopausal Vietnamese women. J. Genet., 90: 1-9.
34. Ye, W.Z., A.F. Reis, D. Dubois-Laforgue, C. Bellanne-Chantelot, J. Timsit and G. Velho, 2001. Vitamin D receptor gene polymorphisms are associated with obesity in type 2 diabetic subjects with early age of onset. Eur. J. Endocrinol., 145: 181-186.