Association of Vitamin D Receptors Genes Polymorphism (*Apa I*, and *Taq I*) with type 1 diabetes in Saudi Arabia (KSA)

El-Sayed El-Badrawy[*1, 6], Zein S. Ibrahim[2,7], AmalAbedel Aziz[3,8], Mahmoud M Kamel[4], Gaber M Shehab[1,9] and Ayman Kamal[5,10]

Department of Biochemistry [1], Department of Physiology[2], Department of internal medicine[3], Department of Pharmacology and Forensic Medicine[5] , Faculty of Medicine , Taif University, kingdom Saudi Arabia. Faculty of Specific Education, Mansoura University[6], Department of Physiology, Faculty of Veterinary Medicine, Kafr El-Sheikh University[7] , Department of Clinical Pathology, NCI, Cairo University[4], Department of, Clinical Pathology, Assuit University[8], Department of Biochemistry, Faculty of Agriculture, Cairo University[9],Department of Toxicology and Forensic Medicine, Faculty of Veterinary Medicine, Suez Canal University[10], Egypt.
mm.kamel@yahoo.com

Abstract: Type 1 diabetes mellitus (TIDM) results from an immune-mediated destruction of insulin-producing-cells in the pancreatic islets of Langerhans. There are clear differences in immunogenetic predisposition to type1 diabetes among countries. Studies have indicated that vitamin D supplementation in early childhood decreases the risk of TIDM. Vitamin D exerts its action via the nuclear vitamin D receptor (VDR), which shows an extensive polymorphism. VDR gene polymorphisms have been associated with altered gene expression or gene function. Four single nucleotide polymorphisms (SNPs) in the VDR gene produce variation in four recognition sites. These recognition sites variants include *Fok* I, *Bsm* I, *Apa* I and *Taq* I. This study was conducted to investigate the relationship between VDR gene polymorphisms and the incidence of TIDM in Saudi people living in Taif region. *Apa* I recognition site was found in low frequency in diabetic patient (7/37)18.9% while, its frequency was high (8/14) 57.1% among normal children. *Taq* I has two recognition sites. The first was found at nucleotide number 293 that was found in a frequency of (1/14) 7.1% in normal non-diabetic individuals while it was detected in (7/37) 18.9% in diabetic patients. The second *Taq* I recognition site was found at nucleotide number 494 without any differences between diabetic and normal individuals. This study indicates that there is an association between VDR genetic polymorphism and incidence of TIDM in Saudi people live in Taif region.

[El-Sayed El-Badrawy, Zein S. Ibrahim, AmalAbedel Aziz, Mahmoud M Kamel, Gaber M Shehaband, Ayman Kamal. **Association of Vitamin D Receptors Genes Polymorphism (*Apa I*, and *Taq I*) with type 1 diabetes in Saudi Arabia (KSA).** *Life Sci J* 2013; 10(3): 1555-1562] (ISSN: 1097-8135). http://www.lifesciencesite.com. 234

Keywords: Vit.D receptors – Polymorphism – Type 1 diabetes

Introduction

Type 1 diabetes mellitus (TIDM) is the most common form of diabetes in childhood and it is characterized by the destruction of pancreatic beta cells resulting in the absence of insulin secretion thus requiring exogenous insulin for survival (Sloka *et al., 2*010) The activation of autoreactive lymphocytes and the cytokine induced apoptosis of pancreatic-cells play a major role in the etiology of type 1 diabetes. There are clear differences in the immunogenetic predisposition to type 1diabetes between countries and the disease incidence seems to vary along with the differences in the predisposition (Atkinson *et al.,* 1994). Global incidence of diabetes mellitus has been increasing in recent decades; there are strong differences between different geographical areas and population groups (Turpeinen *et al.*, 2003). 1,25-Dihydroxyvitamin D3 [1, 25 (O H) 2D3] inhibits lymphocyte activation and affects other elements of the immune system, such as cytokine and immunoglobulin production, as well as major histocompatibility complex (MHC) class II and the cluster differentiation 4 (CD-4) expression (Thomass et, 1994; Pani*et al.,* 2000). Studies in humans have indicated that vitamin D supplementation in early childhood decreases the risk of T1DM and the intake of vitamin D in pregnancy may prevent the appearance of islet autoantibodies in the offspring (Fronczak *et al.,* 2003). Moreover, supplementing infants with vitamin D was suggested to be safe and effective strategy for reducing the risk of T1DM (Bener *et al.,* 2008). Vitamin D exerts its action via the nuclear vitamin D receptor (VDR), which shows an extensive polymorphism. The VDR belongs to the steroid receptor super-family and is widely expressed in many cell types, including lymphocytes, macrophages, and pancreatic -cells (Walters, 1992). VDR is located in the q13 region of chromosome 12 (Bid *et al.,* 2005). Polymorphisms within the VDR gene have been associated with altered gene expression or gene function (Van Etten, 2007). The role of VDR polymorphisms in T1DM pathogenesis has been unclear. Several studies have suggested association between one or more of these SNPs and TIDM

((McDermott et al., 1997;Pani *et al*., 2000; Ban *et al*., 2001;Mohammadnejad *et al*., 2012) but others have failed to confirm this finding (Turpeinen*et al.,* 2003). Recently this inconsistency was attributed to the environmental factors that potentially interfere with the VDR genotype (Ponsonby *et al.,* 2008). The interactions of the genetic background with the development of TIDM are well documented in various populations as the incidence of childhood T1DM is known to vary widely between and within countries.

It is known that type 1 diabetes is a multi-factorial disease, with genetic and environmental factors that could explain the incidence rates that have been found in different ethnic groups and countries. Taking into consideration the environmental influence on development of this disease and its relation with genetic factors, we examined the VDR polymorphisms that interact with vitamin D.

This study was conducted to investigate the relationship between VDR gene polymorphisms and the incidence of TIDM in Saudi people living in Taif region.

2. Material and Methods:

Ethical approval:

This study was approved by the ethical committee of Faculty of Medicine, Taif University.

Patients:

This study included 37 patients with TIDM included 24 males and 13 females with an age range of 3 to 15 years. Among the patients, 2 cases (5 %) showed renal complications, fourteen healthy persons (control group) were recruited for this study. Cases were selected from the Children hospital in Taif city.

Full clinical examination:

Full case history, including the onset of disease, insulin treatment, blood sugar level and complication of the disease were recorded. Data about age, sex, weight, height, liver, kidney, neurological & spleen, eye examination were collected

Sampling:

Three mL of venous blood were withdrawn from each patient at fasting time and postprandial by sterile heparinized tubes. These samples were used for genomic DNA extraction, and estimation of Hb A1C (was measured by high-performance liquid chromatography (normal range, 3.5–6.0%), as well as fasting and postprandial C-peptide, fasting and postprandial blood glucose level was measured using an immunofluorimetric assay with detection limit of 0.15 ng/ml.

Separation of peripheral blood polymorph nuclear leukocytes (PMNs).

PMNs were separated using RPMI solutions and Ficoll-PM (Sigma-Aldrich) according to the manufacturer's instruction

DNA extraction and PCR.

Genomic DNA was extracted from peripheral blood polymorph nuclear (PMNs) cells using a genomic DNA Isolation System (KomaBioteck. Inc., Seoul, Korea). DNA samples concentration and quality were detected using spectrophotometer at 260/280nm. PCR was performed using Taq/Apa-for (5′ -CAG AGC ATG GAC AGG GAG CAA-3′) and Taq/Apa-rev (5′ -G C A ACT CCT CAT GGC TGA GGT CTC-3′) primers that flank a 740-bp fragment of Vit D intron 8/exon 9 as previously described (Pani *et al*., 2000). The PCR conditions included an initial denaturation for 5 min at 94°C, 35 cycles each of which consisted of denaturation at 94°C for 1 min, annealing at 60°C for 1 min and extension at 72°C for 1 min, and final extension for 7 min at 72°C.

Single nucleotide polymorphisms of the Vit D by direct sequencing:

PCR products were subjected to electrophoresis in 1% agarose gel with ethidium bromide staining. VDR specific bands were excised and purified with the QIA quick gel extraction kit (KomaBioteck, Seoul, Korea). Purified PCR products were sequenced directly using specific primer pairs (Macrogen, Korea). Different sequences of the VDR gene were submitted to GenBank (accession numbers KF054040-KF054055).

Multiple sequence analysis and phylogenetic tree:

Comparative analyses were performed using the CLUSTAL W multiple sequence alignment program, Mega 4.1 (Kumar *et al.,* 2001). VDR sequences obtained in the current study were used for the alignments. The phylogenetic tree was constructed by using the neighbor-joining method with Kimura two-parameter distances by using the Mega 4.1. The reliability of internal branches was assessed by 1000 bootstrap replications and the p-distance substitution model.

Inclusion criteria in this study: a) age under 15 years, b) the diabetic patients were considered as affected by T1DM according to American Diabetes Association criteria (Report ADA, 2003).

Statistical analysis:

Data were analyzed using SPSS, version 11.5 statistical software. Comparisons of genotype frequencies between groups were performed using the t-test. The chi-squared test was used for analysis of the difference between the 2 groups. P-value < 0.05 was considered significant.

3. Results and discussion:

Patients were studied at diagnosis and during the course of treatment. The study included 24 males (66%) and 13 females (34%), with an age range of 3 to 15 years, a mean of 8.7 ±4.7 and median 8.5 years. In 37 patients, 2 cases (5%) showed renal complication, 1 case (2%) with eye complication, 6 cases (16%) with uncontrolled TIDM. (VDR) is known to modulate cell

proliferation and differentiation, and calcium absorption from the gut. The action of VDR is regulated by vitamin D, parathyroid hormone, growth factors and protein kinase A. Defects in the VDR gene could modulate the metabolism of calcium thus increasing the risk of developing different diseases including calcium stones, prostate cancer, diabetes, osteoporosis, and many others (Bid *et al*., 2005). The active form of vitamin D, 1,25-Dihydroxyvitamin D, performs its effects via the VDR. Single nucleotide polymorphisms (SNPs) in the VDR gene have been investigated, Apa I A>a (rs7975232), and Taq I T>t (rs731236). The *Apa* I a allele corresponds to a T→G transition and the *Taq* I t allele results in a silent T→C transition in intron 8 and exon 9 respectively (Panierakis*et al.,* 2009). The PCR amplicon used in the reaction, 740 bp of the gene, flanked both the *Apa* I and *Taq* I (designated as rs7975232 and rs731236 SNP, respectively) polymorphic sites. In the present study, we have reported the distribution of VDR (*Taq*-I and *Apa*-I) genotypes in diabetic Saudi patients and compared them with the genotypes in control. *Apa*I recognition site (GGGCC/C) was found at nucleotide number 213 (Suppl 1) in (7/37)18.9% for aa in diabetic patient while it was found among normal children with high frequency; (8/14) 57.1%. The rest of individuals showed GTGCCC motif (Suppl 1, Table 1). *Tqa*I recognition sites (T/CGA) at nucleotide number 494 was found in amplicon from all tested individuals however, a second site was found at nucleotide number 293 that was presented only in some individuals (Suppl. 1). We obtained allelic frequencies of (81.1% vs 18.9%), (42.9% vs 57.1%), for (A vs a) in diabetic patients and control individuals respectively, while (81.1% vs 18.9%), (92.9% vs 7.7%) for (T vs t) alleles in diabetic patients and control individuals respectively (Table 1). The *Taq*I polymorphism results in a silent mutation in the VDR gene (Nishimura *et al.,* 1994), which would therefore not be expected to alter VDR function. The *Apa* I site is located within an intron of the VDR gene. Alterations in intronic sequences may influence protein expression (Mocharla *et al.,* 1997). Four common single SNPs in the VDR gene have been studied intensively (Zmuda *et al.,* 2000): *Fok*I T>C (rs10735810), *Bsm*I A>G (rs1544410), *ApaI* G>T (rs7975232), and *Taq*I C>T (rs731236). These SNPs have been screened for the association with various human traits and diseases (Zmuda *et al*., 2000). Several studies reported association of type 1 diabetes with one of these SNPs (Bonakdaran, *et al.,* 2012 and Mohammadnejad *et al.,* 2012), however, the reported associations were inconsistent among different studies (McDermott *et al*., 1997; Pani *et al*., 2000; Koeleman *et al*., 2002; Guja *et al*., 2002; Fassbender *et al*., 2002; Turpeinen *et al.,* 2003). Here we report a study of association between SNPs in the VDR gene region and type 1 diabetes and their *Apa* I and *Taq*I haplotypes to be associated with type 1 diabetes in the Saudi population. SNPs of *Apa* I and *Taq*I with linkage disequilibrium were detected in the current study.

The VDR locus has been studied for the association with the susceptibility to immune-mediated diseases including T1D, but findings have often been contradictory among different populations worldwide (McDermott *et al*., 1997; Bianco *et al*., 2004; Uitterlinden *et al*. 2004; Guo *et al*., 2006; Ban *et al*., 2007). These could be due to ethnic differences, diverse genetic or environmental factors involved in the pathogenesis of T1D. The VDR polymorphism may reflect linkage disequilibrium and act as marker for functional variants that affect expression levels of VDR rather than being the disease-affecting locus. *Taq*I is a silent SNP in exon 9, however, *Apa* I is located in the intron between exons 8–9 and does not affect VDR protein structure (Obi-Tabot *et al*., 2000; Uitterlinden *et al*., 2004). In addition to the allelic variation in relation to *Apa*I and *Taq*I recognition sites, deduced nucleotide alignment and phylogenetic tree analysis revealed the presence of difference in nucleotide sequences of the tested individuals (Fig.1, Suppl 1). The observed nucleotide substitutions possessed no uniform distribution among different diabetic and control individuals however, the impact of these substitutions on the gene structure and functions are unknown and need further investigation. Our results suggested that the frequency and distribution of the VDR polymorphisms in diabetic Saudi patients and normal persons in Taif were substantially different from other populations. Thus the data suggest an impact of ethnicity and provide a basis for future epidemiological and clinical studies. Conflicting results were found regarding the correlation of Vitamin D receptor and *Bsm*1, *Fok*1, *Apa*1, and *Taq*1 genotype. Some studies showed that the distributions were not different between patients with diabetes and control groups (GogasYavuz *et al.*, 2011).

The apparent discrepancies between this and other studies could be a result of the effect of ethnic differences related to the distribution of *VDR* polymorphisms in these populations, as well as to interactions with other genetic or environmental factors involved in the pathogenesis of type 1 diabetes mellitus (Mohammadnejad *et al.,* 2012)

It seems that environmental factors that influence levels of active vitamin D in humans are complex and a significant difference exists between vitamin D functions and VDR polymorphisms (Bonakdaran *et al.,* 2012).

Interestingly, environmental factor(s) were described to alter the risk associated with VDR SNPs (Zittermann, 2003). This study has been carried out on TID patient and healthy control individuals living in Taif city that is characterized by high altitude situation thus possessing different environmental factor in comparison to other locations in Saudi Arabia. The interaction of this special environmental condition with the genetic constituents may increase or decrease the incidence of TID among Taif population in comparison to other sea level region in KSA which was out of the scope of this study and still need to be elucidated

On conclusion, the present study showed variability in the VDR gene which was recorded on the basis of SNPs of the basic core sequence. There were differences in allele frequency and distribution of genotypes of VDR (*Taq*-I and *Apa*-I) in diabetic patient than control. The samples were taken from Taif city that is characterized by high altitude situation thus possessing different environmental factor in comparison to other locations in Saudi Arabia.

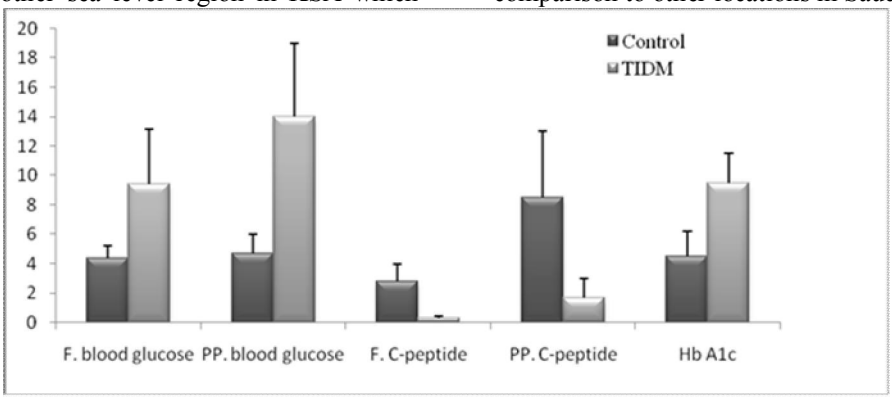

Figure (1). The clinical and laboratory data of the patients and the control (Fasting and postprandial blood glucose (mmol/L), C-peptide (ng/ml) and HbA1c (%)

Table (1): VDR polymorphism

	Site one For Taq I	Site two For TaqI	ApaI
Control	**14**	**1/14**	**8/14**
Diabetes	**37**	**7/37**	**7/37**

Fig.(2): Phylogenetic tree of VDR sequences of the Saudi nationals generated by neighbour-joining analysis. The robustness of individual nodes of the tree was assessed using a bootstrap of 1000 replications of bootstrap re-sampling of the originally-aligned nucleotide sequences. Scale bar represents 0.002 nucleotide substitutions.

Supplement 1: **Deduced nucleotide sequence of different VDR from Saudi nationals.** Dots mean homologous nucleotides. The recognition sites of the restriction enzymes ApaI and TaqI are boxed.

D1_2Taq CAGAGCATGG ACAGGGAGCA AGGCCAGGCA GGGACAGGGC CAGGTGCGCC CATGGAAGGA CCTAGGTCTG GATCCTAAAT GCACGGAGAA GTCACTGGAG GGCTTTGGGG CCAGGCAGTG [120]

#D3 .... [120]
#D4_Apa .... [120]
#D5_Apa .... [120]
#D7 .... [120]
#D9_Apa .... [120]
#D14_Apa .... [120]
#D23_Apa .... [120]
#D28 .....C.... .... [120]
#D29 .... [120]
#D30_2Taq .... [120]
#C1_Apa .... [120]
#C6 .... [120]
#C7_Apa_2_Taq .... [120]
#C9_Apa ....C. .... [120]
#C10 ....C. .... [120]
#C13_Apa .... [120]

GGGCCC(ApaI)

#D1_2Taq GTATCACCGG TCAGCAGTCA TAGAGGGGTG GCCTAGGGGG TGCTGCCGTT GAGTGTCTGT GTGGGTGGGG GGTGGTGGGA TTGAGCAGTG AGGTGCCCAG CTGAGAGCTC CTGTGCCTTC [240]

#D3 .... C..... .... [240]
#D4_Apa .... C..... ....G..... .... [240]
#D5_Apa .... C..... ....G..... .... [240]
#D7 .... [240]
#D9_Apa ....G..... .... [240]
#D14_Apa ....G..... .... [240]
#D23_Apa ....G..... A..... .....G [240]
#D28 .... G..... ..... [240]
#D29 .... C..... .... [240]
#C1_Apa .... C..... ....G..... .... [240]
#C6 .... [240]
#C7_Apa_2_Taq ....G..... .... [240]
#C9_Apa ....G..... .... [240]
#C10 .... [240]
#C13_Apa ....G..... .... [240]

TCGA(TaqI)

#D1_2Taq TTCTCTATCC CCGTGCCCAC AGATCGTCCT GGGGTGCAGG ACGCCGCGCT GATCGAGGCC ATCCAGGACC GCCTGTCCAA CACACTGCAG ACGTACATCC GCTGCCGCCA CCCGCCCCCG [360]

#D3 ....T..... .... [360]
#D4_Apa ....T..... .... [360]
#D5_Apa ....G..... ....T..... .... [360]
#D7 ....T..... .... [360]
#D9_Apa ....T..... .... [360]
#D14_Apa ....T..... .... [360]
#D23_Apa ....T..... .... [360]
#D28 ....C. ....T..... ....G..... ..... [360]
#D29 ....T..... .... [360]
#C1_Apa ....T..... .... [360]
#C6 ....T..... .... [360]
#C7_Apa_2_Taq .... [360]
#C9_Apa ....T..... .... [360]

#C10 ....T..... .... [360]
#C13_Apa ....T..... ....G.. .... [360]

#D1_2Taq GGCAGCCACC TGCTCTATGC CAAGATGATC CAGAAGCTAG CCGACCTGCG CAGCCTCAAT GAGGAGCACT CCAAGCAGTA CCGCTGCCTC TCCTTCCAGC CTGAGTGCAG CATGAAGCTA [480]
#D3 .... [480]
#D4_Apa .... [480]
#D5_Apa .... [480]
#D7 .... [480]
#D9_Apa ....A..... .... [480]
#D14_Apa .... [480]
#D23_Apa .... [480]
#D28 .... [480]
#D29 .... [480]
#C1_Apa .... [480]
#C6 .... [480]
#C7_Apa_2_Taq .... [480]
#C9_Apa .... [480]
#C10 .... [480]
#C13_Apa .... [480]

TCGA(*TaqI*)
#D1_2Taq ACGCCCCTTG TGCTCGAAGT GTTTGGCAAT GAGATCTCCT GACTAGGACA GCCTGTGGCG GTGCCTGGGT GGGGCTGCTC CTCCAGGGCC ACGTGCCAGG CCCGGGGCTG GCGGCTACTC [600]
#D3 .... [600]
#D4_Apa .... [600]
#D5_Apa .....G... .... [600]
#D7 .... [600]
#D9_Apa .... [600]
#D14_Apa .... [600]
#D15 .... [600]
#D23_Apa .... [600]
#D28 .... [600]
#D29 .... [600]
#C1_Apa .... [600]
#C6 .... [600]
#C7_Apa_2_Taq .... [600]
#C9_Apa .... [600]
#C10 .... [600]
#C13_Apa .... [600]

#D1_2Taq AGCAGCCCTC CTCACCCCGT CTGGGGTTCA GCCCCTCCTC TGCCACCTCC CCTATCCACC CAGCCCATTC TCTCTCCTGT CCAACCTAAC CCCTTTCCTG CGGGCTTTTC CCCGGTCCCT [720]
#D3 .... [720]
#D4_Apa ....G... .... [720]
#D5_Apa .... [720]
#D7 .... [720]
#D9_Apa .... [720]
#D14_Apa .... [720]
#D15 .... [720]
#D23_Apa .... [720]
#D28 .... [720]
#D29 .... [720]
#C1_Apa .... [720]
#C6 .... [720]
#C7_Apa_2_Taq .... [720]

#C9_Apa .... [720]
#C10 .... [720]
#C13_Apa .... [720]

#D1_2Taq TGAGACCTCA GCCATGAGGA GTTGC [745]
#D3 .... [745]
#D4_Apa .... [745]
#D5_Apa .... [745]
#D7 .... [745]
#D9_Apa .... [745]
#D14_Apa .... [745]
#D15 .... [745]
#D21 .... [745]
#D23_Apa .... [745]
#D28 .... [745]
#D29 .... [745]
#C1_Apa .... [745]
#C6 .... [745]
#C7_Apa_2_Taq .... [745]
#C9_Apa .... [745]
#C10 .... [745]
#C13_Apa .... [745]

References:

1. **Atkinson MA, MacLaren NK. (1994)**. Mechanisms of disease: thepathogenesis of insulin-dependent diabetes mellitus. N Engl J Med, 331:1428-1436.
2. **Ban, Y., Taniyama, M., Yanagawa, T. (2001**). Vitamin D receptor initiation codon polymorphism influences genetic susceptibility to type 1 diabetes mellitus in the Japanese population, BMC Med. Genet. 2 7.
3. **Bener A, Alsaied A, Al-Ali M, Hassan AS, Basha B, Al-Kubaisi A, Abraham A, Mian M, Guiter G, Tewfik I. (2008).**Impact of lifestyle and dietary habits on hypovitaminosis D in type 1 diabetes mellitus and healthy children from Qatar, a sun-rich country. Ann Nutr Metab. 53(3-4):215-22.
4. **Bianco, M.G., Minicucci, L., Calevo, M.G. (2004).** Vitamin D receptor polymorphisms: are they really associated with type 1 diabetes, Eur. J. Endocrinol. 151, 641–642.
5. **Bid HK, Mishra DK, Mittal RD. (2005).** Vitamin-D receptor (VDR) gene (Fok-I, Taq-I and Apa-I) polymorphisms in healthy individuals from north Indian population. Asian Pac J Cancer Prev. 6(2):147-52.
6. **Bonakdaran, S.; M.R. Abbaszadegan; E. Dadkhah and M. Khajeh-Dalouie. (2012).** Vitamin D receptor gene polymorphisms in type 1 diabetes mellitus: a new pattern from Khorasan province, Islamic Republic of Iran. Eastern Mediterranean Health Journal (EMHJ), Vol. 18, 6, 614-619
7. **Fassbender WJ, Goertz B, Weismuller K, Steinhauer B, Stracke H, Auch D, Linn T, Bretzel RG. (2002).** VDR gene polymorphisms are overrepresented in German patients with type 1 diabetes compared to healthy controls without effect on biochemical parameters of bone metabolism. HormMetab Res 34:330–337
8. **Fronczak, C.M.; A.E. Baron; H.P. Chase (2003).** In utero dietary exposures and risk of islet autoimmunity in children, Diabetes Care 26, 3237–3242.
9. **Gogas Yavuz D, Keskin L, Kıyıcı S, Sert M, Yazıcı D, Sahin I, Yüksel M, Deyneli O, Aydın H, Tuncel E, Akalın S. (2011).**Vitamin D receptor gene BsmI, FokI, ApaI, TaqI polymorphisms and bone mineral density in a group of Turkish type 1 diabetic patients.ActaDiabetol. (4):329-36.
10. **Guja C, Marshall S, Welsh K, Merriman M, Smith A, Todd JA, IonescuTirgoviste C. (2002).**The study of CTLA-4 and vitamin D receptor polymorphisms in the Romanian type 1 diabetes population. J Cell Mol Med 6:75–81
11. **Guo, S.W., Magnuson, V.L., Schiller, J.J. (2006).** Meta-analysis of vitamin D receptor polymorphisms and type 1 diabetes: a HuGE review of genetic association studies, Am. J. Epidemiol. 164, 711–724.
12. **Koeleman BP, Valdigem G, Eerligh P, Giphart MJ, Roep BO. (2002).** Seasonality of birth in

patients with type 1 diabetes (Letter). Lancet 359:1246–1247 ,

13. **Kumar S, Tamura K, Jakobsen IB, Nei M. (2001).** Molecular evolutionary genetics analysis software. Bioinformatics, 17:1244-1245 .
14. **McDermott MF, Ramachandran A, Ogunkolade BW, Aganna E, Curtis D, Boucher BJ, Snehalatha C, Hitman GA. (1997).** Allelic variation in the vitamin D receptor influences susceptibility to IDDM in Indian Asians. Diabetologia 40:971–975.
15. **Mohammadnejad Z, Ghanbari M, Ganjali R, Afshari JT, Heydarpour M, Taghavi SM, Fatemi S, Rafatpanah H.(2012)**. Association between vitamin D receptor gene polymorphisms and type 1 diabetes mellitus in Iranian population. Mol Biol Rep;39(2):831-837.
16. **Mocharla H, Butch AW, Pappas AA, Flick JT, Weinstein RS, De Togni P, Jilka RL, Roberson PK, Parfitt AM, Manolagas SC. (1997).** Quantification of vitamin D receptor mRNA by competitive polymerase chain reaction in PBMC: lack of correspondence with common allelic variants. J Bone Miner Res 12:726–733
17. **Nishimura A, Shinki T, Jin CH, Ohyama Y, Noshiro M, Okuda K, Suda T. (1994).**Regulation of messenger ribonucleic acid expression of 1 alpha, 25-dihydroxyvitamin D3-24-hydroxylase in rat osteoblasts. Endocrinology 134:1794–1799.
18. **Obi-Tabot, E.T., Tian, X.Q., Chen, T.C., Holick, M.F. (2000).** A human skin equivalent model that mimics the photoproduction of vitamin D3 in human skin, In Vitro Cell Dev. Biol. Anim. 36, 201–204.
19. **Pani MA, Knapp M, Donner H, Braun J, Baur MP, Usadel KH, Badenhoop K. (2000).** Vitamin D receptor allele combinations influence genetic susceptibility to type 1 diabetes in Germans. Diabetes 49:504–507.
20. **Panierakis C, Goulielmos G, Mamoulakis D, Petraki E, Papavasiliou E, Galanakis E. (2009**). Vitamin D receptor gene polymorphisms and susceptibility to type 1 diabetes in Crete, Greece. ClinImmunol. 133(2):276-81.
21. **Ponsonby, A.L.; A. Pezic; J. Ellis; R. Morley; F. Cameron; J. Carlin, T. Dwyer (2008).** Variation in associations between allelic variants of the vitamin D receptor gene and onset of type 1 diabetes mellitus by ambient winter ultraviolet radiation levels: a meta-regression analysis, Am. J. Epidemiol. 168, 358–365.
22. **Sloka S, Grant M, Newhook LA. (2010).**The geospatial relation between UV solar radiation and type 1 diabetes in New foundland. Acta Diabetol. Mar;47(1):73-8
23. **Thomasset M. (1994).**Vitamin D and the immune system.PatholBiol 42:163–172.
24. **Turpeinen H, Hermann R, Vaara S, Laine AP, Simell O, Knip M, Veijola R, Ilonen J. (2003).** Vitamin D receptor polymorphisms: no association with type 1 diabetes in the Finnish population. Eur J Endocrinol 149:591–596.
25. **Uitterlinden, A.G., Fang, Y., Van Meurs, J.B., Pols, H.A., Van Leeuwen, J.P. (2004).**Genetics and biology of vitamin D receptor polymorphisms, Gene 338, 143–156.
26. **Van Etten E, Verliden L, Giulietti A, Ramos-Lopez E, Branisteanu DD, Ferreira GB, (2007).** The vitamin D receptor gene FokI polymorphism: Functional impact on the immune system. Eur J Immunol;37:395–405.
27. **Walters MR (1992).**Newly identified actions of the vitamin D endocrine system.Endocr Rev 13:719–764.
28. **Zittermann A. (2003).** Vitamin D in preventive medicine: are we ignoring the evidence? Br J Nutr 89:552–572.
29. **Zmuda JM, Cauley JA, Ferrell RE. (2000).** Molecular epidemiology of vitamin D receptor gene variants. Epidemiol Rev 22:203–217.

8/11/2013