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VDBP, *VDR* mutations and other factors related with vitamin D metabolism may be associated with type 1 diabetes mellitus

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Abstract: Type 1 diabetes mellitus (T1DM) is an insulin dependent autoimmune disorder resulting the progressive destruction of pancreatic beta cells. Another possible factor considered to be related with T1DM is vitamin D deficiency. Therefore in this study it was aimed to investigate the associations between T1DM, vitamin D binding protein (*VDBP)* and vitamin D receptor (*VDR*) gene mutations which are related with vitamin D metabolism. Fifty five T1DM paitents and 40 healthy volunteers were recruited to the study. *FokI* (rs2228570), *BsmI* (rs1544410) mutations in *VDR*; rs4588 and rs7041 polymorphisms in *VDBP* were investigated with real-time polymerase chain reaction (RT-PCR). Other risk factors related with T1DM were also investigated. Results were evaluated statistically. Statistically significant relations were found in glucose, HbA1c, TSH, higher 25[OH]D, free vitamin D, calcium, albumin, log25[OH]D, retinopathy, higher than 30 mg/day microalbuminuria in T1DM patients. Also statistically significant association was found between C allele in *Fok*1 and T1DM in patients. When the relation between the risk factors and mutations were investigated, it was found that VDBP, free vitamin D and bioactive vitamin D were significantly associated with rs7041 mutation in *VDBP* whereas HDL was significantly associated with rs2228570 mutation in *VDR*. Other studies with larger data sets may demonstrate more reliable statistical results to rule out genotype-phenotype correlations of the disease.

Key words: T1DM; *VDR*; *VDBP*; RT-PCR; Vitamin D deficiency.

Introduction

Type 1 diabetes mellitus (T1DM) is an insulin dependent autoimmune disease caused by the progressive destruction of pancreatic beta cells due to interactions between genetically susceptible genes and environmental exposure in individuals (1). The aetiology of T1DM is not totally described since the involvement of both genetic and environmental factors, also the interactions between those two. Today, T1DM is increasing worldwide and one of the research area is to obtain a significant role for environmental influences via gene polymorphisms (2).

Vitamin D (calciferol) is a steroid hormone which has been linked to the pathogenesis of various clinical conditions, like T1DM (3,4). Data from 2008 suggested that at least one billion individuals with vitamin D deficiency suffer from lack of vitamin D worldwide (4). Studies showed that vitamin D deficiency has been involved in several processes such as inflammation, endothelial dysfunction and up-regulation of the renin-angiotensin-aldosterone system (RAAS) which those are thought to be in association with the type 1 diabetes (5). Considering vitamin D metabolites are important in risk of T1D, it seems highly expected that there might be an association between risk of T1D and genes in the vitamin D pathway that increase or decrease the level of the active metabolite (2). For vitamin D, deficiency define as a 25(OH)D below 20 ng/ mL, insufficiency as a 25 (OH)D of 21 – 29 ng/mL, and sufficiency as a 25 (OH)D of 30 – 100 ng/mL (6). Vitamin D deficiency should probably be effective in individuals at high risk of developing T1DM, particularly in early life (7).

Vitamin D is the collective name for cholecalciferol (D_3) and ergocalciferol (D_2) which are precursors of the active vitamin D receptor (VDR) ligand (8). The VDR on chromosome 12q12–q14 is a nuclear transcription factor that regulates the expression of approximately 500 genes including the transcription of vitamin-D sensitive genes (9, 10). Two of the most commonly studied VDR polymorphisms are *Fok1* and *Bsm1* which are referred to as letters (or as nucleotides), such as *Fok1* F/f (rs2228570 C/T, known also as rs10735810), *Bsm1* B/b (rs1544410, A/G) (2). *Fok*1 polymorphism in exon 2 is characterized by the presence of either two ATG start codons in the long f-VDR or only one start codon due to a T-to-C substitution in the short F-VDR. This leads to a 3-amino acids (aa) shorter protein (with 424aa instead of 427aa) (11, 12). *Bsm*I polymorphism, in intronic region between exons 8 and 9, takes place near the 3′ end of the *VDR* gene and is strongly linked with 3 poly(A) microsatellite repeat in the 3′ untranslated region, which may influence *VDR* mRNA stability, instead of affecting VDR protein structure (1). The role of *VDR* polymor-

phisms in the pathogenesis of TIDM has not been clarified. Studies have shown that vitamin D in humans is an indispensable component of vitamin D functions and *VDR* polymorphisms (13). Similar confusing data exist for linkage between T1DM and polymorphisms in the genes encoding enzymes with central roles in vitamin D metabolism, like vitamin D binding protein (VDBP). By VDBP, Vitamin D is transferred from the skin or intestine into blood stream where it remains bound while circulating. VDBP gives vitamin D into liver for activation. Liver is the place that the 25-hydroxy-vitamin D (25 (OH) D) is synthesized from vitamin D and re-fed to VDBP in circulation (8).

The *VDBP* which is encoded by the GC gene in chromosome 4, is the primary transport of 1.25 $(OH)_2D_3$. VDBP is thought to be had immune regulatory properties itself. Specific *VDBP* genotypes are associated with the detection of IA-2 antibodies and have shown a correlation between *VDBP* polymorphisms and T1DM (4). *VDBP* has three common phenotypic alleles which differ from each other by glycosylation patterns and amino acid substitutions in the *VDBP* gene. These polymorphisms are known as rs4588 and rs7041 which are both located in coding region. rs7041 (G>T) encodes for the glutamic acid to aspartic acid conversion, while rs4588 (C>A) encodes for the threonine to lysine (14).

The need for interventional well designed studies with vitamin D substitution for determining the role of vitamin D and its supplementation in preventing/ understanding type 1 diabetes is crucial. Therefore, the main goal of this study is to determine the role of genetic and environmental risk factors which are thought to be in an association between vitamin D metabolism and T1DM.

Materials and Methods

Study population

Totally, 55 patients who were diagnosed as Type 1 diabetes mellitus and 40 healthy individuals without any diseases were included from Marmara University Hospital, Endocrinology and Metabolic Diseases Clinic from July 2016-Janauary 2017. Sex, age, BMI, waist circumference, duration of diabetes, glucose, thyroid stimulating hormone (TSH), high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, vitamin D binding protein (VDBP) level, parathyroid hormone (PTH), calcium, phosphorus, albumin, vitamin D, free vitamin D, bioactive vitamin D levels were measured and recorded into digital media. Subjects who are pregnant or lactating women, and those currently on vitamin D supplementation were excluded from the analysis.

Free and bioactive vitamin D levels were calculated with the formula below (15). In this formula, the calculated correlation coefficient value is 0.925 which was measured by ultrafiltration method.

$$\text{Free 25 (OH)D} = \frac{-b+\sqrt{b^2-4ac}}{2a}$$

a= K(DBP)*K(ALB)*[ALB]+K (DBP)
b= K(DBP)*[DBP]-
K(DBP)*[DTOTAL]+K(ALB)*[ALB]+1
c= -[DTOTAL]

Bioactive D: [K(ALB)*[ALB]+1]*[Free (25(OH)D]
[DBP]: D binding protein, [ALB]: Albumin, *(K(ALB): 6×10^5, K(DBP):* 7×10^8)

The present study protocol conforms to the Declaration of Helsinki and was approved by the Institutional Ethics Committee of Yeditepe University, Istanbul, Turkey. Written informed consent was obtained from all patients prior to their participation in the study.

Blood sampling and genotyping

DNA was extracted from 200 µL of blood using commercially available kits (Qiagen, Germany) according to manufacturer's instructions. DNA purity and concentration were determined by NanoDrop spectrophotometer (Thermo Fisher Scientific, USA). Real-time PCR reactions for rs2228570, rs1544410 in *VDR*, rs4588 and rs7041 in *VDBP* were carried out on 7500 Fast Real-Time PCR System (Applied Biosystems). The reaction was performed by the manufacturer's instructions.

Statistical analysis

Statistical analyses were performed using IBM SPSS (Statistical Package of Social Sciences, Version 23.0. Armonk, NY: IBM Corp. Descriptive analysis was presented using means ± standard deviations (SD) for continuous data and frequencies and percentages for categorical data. The variables were investigated using Kolmogorov Smirnov test to determine whether they are normally distributed. If the variables are not normally distributed, Mann-Whitney U test was used to compare the two independent groups. Since the variables are not normally distributed, Kruskal-Wallis test were conducted to compare risk factors among genotypes. Mann-Whitney U test was performed to test the significance of pairwise differences using Bonferroni correction adjust for multiple comparisons The Chi-Square and Fisher's exact test, where appropriate, was used to compare the proportions of the groups. Relationships between the variables were determined using Contengency Coefficients. A 5% type-I error level was used to infer a statistical significance.

Results

Study population

Table 1 shows the baseline characteristics of the study population. Significant differences were observed in glucose, HbA1c, TSH, 25[OH]D, free vitamin D, calcium, albumin, log25[OH]D, retinopathy, microalbuminuria >30, vitamin D deficiency >20.

VDR and *VDBP* genotyping

Table 2 shows genetic analyses of cases and controls. When groups were compared with each other, rs2228570 mutations were found statistically significant in cases ($p<0.05$).

Relation between risk factors, *VDR* and *VDBP* mutations

Table 3 shows statistically significant relations between risk factors and mutations.

Table 1. Baseline characteristics of the study population.

Baseline Characteristics		Groups (number of participants)		p values
		Patients(n=55)	Controls (n=40)	
Age		29.8 ± 7.75	28.9 ± 5.29	0.50
BMI		24.29 ± 3.73	27.76 ± 3.99	0.56
Waist circumference (cm)		79.69 ± 10.02	80.48 ± 9.97	0.71
Duration of the disease (year)		10.49 ± 6.9	-	n.e.
Glucose		193.11 ± 98.59	86.78 ± 11.56	<0.001**
HbA1c		8.48 ± 2.14	4.49 ± 0.56	<0.001**
TSH (mIU/L)		2.02 ± 1.17	1.45 ± 0.52	0.002*
HDL (mg/dl)		55.31 ± 13.24	52.98 ± 11.21	0.37
LDL (mg/dl)		113.73 ± 34.48	124.38 ± 34.95	0.14
Triglyceride (mg/dl)		133.89 ± 102.23	111.23 ± 60.84	0.18
25[OH]D (ng/ml)		18.09 ± 10.07	12.77 ± 7.52	0.006*
VDBP (ng/ml)		355.77 ± 241.09	417.65 ± 240.6	0.22
Free vitamin D (pg/ml)		4.92 ± 3.52	3.33 ± 2.229	0.028*
Bioactive vitamin D (ng/ml)		1.95 ± 1.4	1.4 ± 1.37	0.058
PTH (pg/ml)		39.22 ± 16.23	40.89 ± 18.58	0.64
Calcium (mg/dl)		9.56 ± 0.47	9.88 ± 0.46	0.002*
Phosphate (mg/dl)		3.54 ± 0.62	3.4 ± 0.5	0.28
Albumin (g/dl)		4.4 ± 0.36	4.79 ± 0.4	<0.001**
Microalbuminuria		17.5 ± 15.08	-	n.e
LogPTH		1.55 ± 0.19	1.57 ± 0.17	0.59
Log[25OHD]		1.18 ± 0.29	1.04 ± 0.24	0.015*
Retinopathy		15 (27.3%)	0 (0%)	<0.001**
Neuropathy		-	-	n.e.
Gender	Female	29 (52.7%)	21 (52.5%)	0.98
	Male	26 (47.3%)	19 (47.5%)	
Nephropathy	Microalbuminuria<30 mg/day	42 (76.4%)	40 (100%)	0.001*
	Microalbuminuria>30 mg/day	13 (23.6%)*	0 (0%)	
Vitamin D	Deficient	34 (61.8%)	33 (82.5%)	0.074
	Insufficient	15 (27.3%)	6 (15%)	
	Normal	6 (10.9%)	1 (2.5%)	
Vitamin D deficiency 20	<20 deficient	34 (61.8%)	33 (82.5%)	0.029*
	>20 insufficient or normal	21 (38.1%)*	7 (17.5%)	
Vitamin D deficiency 30	<30 insufficient or deficient	49 (89%)	39 (97.5%)	0.23
	>30 normal	6 (10.9%)	1 (2.5%)	
LDL deficiency	<130 mg/dl	41(74.5%)	23 (57.5%)	0.08
	>130 mg/dl	14 (25.5%)	17 (42.5%)	

BMI: Body mass index, HbA1c: Hemoglobin A1c, TSH: Thyroid-stimulating hormone, HDL: High density lipoprotein, LDL: Low density lipoprotein, 25[OH]D: 25-hydroxyvitamin D, VDBP: Vitamin D binding protein, PTH: Parathyroid hormone. *p<0.05, **p<0.001, n.e.: not evaluated.

Discussion

T1DM is an autoimmune disease in which the body's own immune system accidentally attacks and destroys β cells that produce pancreatic insulin. T1DM is usually seen in young, lean individuals, but elderly patients may also be affected (16). It is one of the first endocrine disorders where a potential role for vitamin D was reported. An association between vitamin D and T1DM was reported by Ponsonby et al. who suggested that T1DM may be dependent on vitamin D receptor variants (17). Here, we investigated the role of vitamin D metabolism related polymorphisms which may effect T1DM progression and we found significant association between *Fok*1 and T1DM. This result overlaps with the meta-analysis performed by Sahin et al. showed that there is a statistical significant association for *Fok*1 *ff* polymorphism (CC in our study) in T1DM patients (12). Also another study demonstrated that *BsmI* polymorphism is associated with increased risk of T1DM, particularly in Asian subjects (3). In another study, it was found that, there is an association between the *Bsm*1 and *Fok*1 polymorphisms of *VDR* and risk of T1DM (18). Contrary to these findings, we could not detect any significant relation between *Bsm*1 and T1DM. White people are more likely to have A allele in rs4588 than those reported to be associated with higher VDBP concentrations, as opposed to those with T allele in rs7041 that are shown to

Table 2. Genetic analysis in cases and controls.

Gene, genotype and nucleotide variations	Groups		p values
	Cases (n=55)	Controls (n=40)	
VDBP			
rs4588			
CC	32 (58.2%)	27 (67.5%)	
CA	20 (36.4%)	12 (30%)	0.58
AA	3 (5.5%)	1 (2.5%)	
rs7041			
GG	8 (14.5%)	5 (12.5%)	
GT	27 (49.1%)	15 (37.5%)	0.41
TT	20 (36.4%)	20 (50%)	
VDR			
*Fok*1 (rs2228570)			
TT	4 (7.3%)	10 (25%)	
TC	23 (41.8%)	22 (55%)	**0.003***
CC	28 (50.9%)*	8 (20%)	
*Bsm*1 (rs1544410)			
GG	22 (40%)	13 (32.5%)	
GA	21 (38.2%)	17 (42.5%)	0.76
AA	12 (21.8%)	10 (25%)	

VDBP: Vitamin D binding protein gene, *VDR*: Vitamin D receptor gene. *p<0.05

Table 3. Relation between disease associated risk factors and *VDR*, *VDBP* mutations.

Risk factors and mutations	Mean values for genotypes			p values
rs7041	**GG (n=13)**	**GT(n=42)**	**TT(n=40)**	
VDBP (ng/dl)	212.31 ± 155.7*	362.13 ± 217.55	457.59 ± 259.89*	**0.004***
Free vitamin D (pg/ml)	7.63 ± 4.35*′	3.82 ± 2.97′	3.6 ± 3.16*	**0.003*/0.009′**
Bioactive D (ng/ml)	3.15 ± 1.9*′	1.54 ± 1.18′	1.45 ± 1.18*	**0.004*/0.007′**
rs2228570	**TT (n=14)**	**TC (n=45)**	**CC (n=36)**	
HDL (mg/dl)	46.36 ± 10.58*′	55.53 ± 11.98′	55.92 ± 12.74*	**0.041*/0.049′**

VDBP: Vitamin D binding protein, HDL: High density lipoprotein. *p<0.05, ′p<0.05

be associated with VDBP concentrations (19). In large cohort study, no difference in genotype frequencies of *VDBP* polymorphisms showed a relationship between serum VDBP levels or control subjects versus T1DM patients (20). In this study we could not detect any significant relation between T1DM and *VDBP* mutations however statistically significant relation was found between rs7041 mutation and VDBP concentrations.

T1DM develops as a result of complex interaction between genetic and environmental factors. Since the vitamin D involve in the induction of autoimmune destruction of β-cells, this makes it possible factor for T1DM, especially in early life. Vitamin D deficiency has extensive effects on insulin resistance, beta cell dysfunction and hypertension and is thought to be associated with diabetic complications (21). Contrary to these findings, in our study, the levels of vitamin D were found statistically higher in patients than controls (p=0.029)

Type 1 diabetes mellitus is a chronic autoimmune disease characterized by increased blood glucose levels which is known as hyperglycemia. Therefore, it was expected to find statistical difference between glucose levels of two groups in present study (p<0.001). One of the possible monitoring method in order to determine glucose level is to measuring Hb1Ac. The glycosylated/glycated hemoglobin test (Hb1Ac) performs the most objective and reliable information on long-term glucose control in diabetic patients (22). An increase in glycosylated hemoglobin level causes greater risks of diabetes. Such that, lower HbA1c level was associated with less development or progression of retinopathy and nephropathy which are known as microvascular complications develop during T1DM (23). As we expected, we found Hb1Ac value of patient group as statistically significant (p<0.001).

Diabetic retinopathy is approximately affected 93 million people worldwide. Its pathophysiology is highly related with the interactions between neural and retinal vascular dysfunction (21). It is known that vitamin D deficiency plays a role in the development of diabetic complications such as nephropathy and retinopathy (24). Therefore, our result showing significant value for T1DM patients with retinopathy (%21 of total patient group) overlaps with others. *VDR* polymorphisms have also been linked to retinopathy risk in T1DM (25) but we could not observe any relation. Microalbuminuria is one of the well-known sign of early renal disease (4). A study indicated that paricalcitol therapy, which has been known as vitamin D analogue, significantly reduces urinary albumin excretion (26). In present study, it is claimed that microalbuminuria higher than 30 mg/day was statistically significant in T1DM patients (23,6 %) inde-

pendently from vitamin D. Overlapping with our findings, in a cross-sectional study of 517 patients, vitamin D deficiency was not associated with microalbuminuria or neuropathy (27). Discrepancies between the current findings and these previous findings may be explained by the lower numbers of albuminuria cases in the current study than in the previous ones.

VDBP is a polymorphic, single chain serum glycoprotein. Approximately 90% of 25 (OH) D in the circulation is bound to VDBP, 10% to albumin, less than 1% is found as free form (28). The binding affinity for the D vitamin metabolites to VDBP is 1000 times stronger than that of albumin, and therefore the albumin-bound and free fractions together are biologically available (29). A study found lower concentrations of VDBP in patients with type 1 diabetes when compared with their first-degree relatives and healthy controls (20). Similarly in our study it was found that patients have low VDBP levels than controls. Since VDBP delivers vitamin D to the liver for activation, where 25-hydroxyvitamin D (25[OH]D) is synthesized from vitamin D; it is possible to find significantly lower VDBP related to higher 25[OH]D and its logarithmic transformation form, as it was observed in this study.

Thyroid diseases and diabetes mellitus are the most common endocrine disorders encountered in clinical practice. Thyroid dysfunction prevalence is high in diabetics with the highest prevalence in type 1 female diabetics (31.4%) and lowest prevalence in type 2 male diabetics (6.9%) (30). Here our study subjects were not diagnosed as hypothyroid (TSH>4 mIU/L) or hyperthyroid (0.5-4 mIU/L) patient. However, in present study, the mean serum TSH levels were 2.02 ± 1.17 in the T1DM group and 1.45 ± 0.52 in the control group and significantly higher in the T1DM group ($p = 0.002$).

When the relation between the risk factors and genotypes were investigated, it was found that individuals who carry two T allele for rs7041 have statistically high VDBP, low free vitamin D and bioactive D concentrations. Therefore it was considered that there is a relation between rs7041 mutation and different forms of vitamin D. Similarly, in another study, it was found that low 25(OH)D with diabetes were strong in white adults with the A allele of rs4588 as well as the T allele of rs7041 (19). Also, in another study, it was suggested that A allele of rs4588 is associated with higher VDBP concentrations (15). In contrast to these findings we couldn't found any relation between rs4588 mutation with VDBP and 25(OH)D levels. Another relation between risk factors and mutations was observed for rs2228570 polymorphism. In our study, it was found that individuals who carry two C alleles have high HDL concentrations than other genotypes. This result overlapped with a study indicating that patients with the CC genotype had significantly higher concentrations of HDL-cholesterol than those with the TC genotype or the TT genotype (31).

In conclusion, the present study indicates an association between *VDR* and *VDBP* SNPs and T1DM among Turkish subjects. We suggest that *Fok*1 mutation was significantly associated with T1DM (p=0.003). Also, VDBP, free vitamin D and bioactive vitamin D were significantly associated with rs7041 in *VDBP* whereas HDL were significantly associated with rs2228570 in *VDR*. Yet, still, influence of vitamin D related gene polymorphisms on susceptibility to T1DM deserves further investigations. Meta-analysis includes larger data sets may demonstrate more reliable statistical results to rule out genotype–phenotype correlations of T1DM.

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Conflict of interest
All of the authors have no conflict of interest to declare.

References

1. Cheon CK, Nam HK, Lee KH, Kim SY, Song JS, Kim C. Vitamin D receptor gene polymorphisms and type 1 diabetes mellitus in a Korean population. Pediatr Int 2015;57(5):870-874.
2. Miller KM, Hart PH, de Klerk NH, Davis EA, Lucas RM. Are low sun exposure and/or vitamin D risk factors for type 1 diabetes? Photochem Photobiol Sci 2017;16(3):381-398.
3. Altieri B, Muscogiuri G, Barrea L, Mathieu C, Vallone CV, Mascitelli L, et al. Does vitamin D play a role in autoimmune endocrine disorders? A proof of concept. Rev Endocr Metab Disord 2017.
4. Grammatiki M, Rapti E, Karras S, Ajjan RA, Kotsa K. Vitamin D and diabetes mellitus: Causal or casual association? Rev Endocr Metab Disord 2017;18(2):227-241.
5. Engelen L, Schalkwijk CG, Eussen SJ, Scheijen JL, Soedamah-Muthu SS, Chaturvedi N, et al. Low 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3 levels are independently associated with macroalbuminuria, but not with retinopathy and macrovascular disease in type 1 diabetes: the EURODIAB prospective complications study. Cardiovasc Diabetol 2015;14:67.
6. Griz LH, Bandeira F, Gabbay MA, Dib, SA, Carvalho EF. Vitamin D and diabetes mellitus: an update 2013. Arq Bras Endocrinol Metabol 2014;58(1):1-8.
7. Muscogiuri G, Mitri J, Mathieu C, Badenhoop K, Tamer G, Orio F, et al. Mechanisms in endocrinology: vitamin D as a potential contributor in endocrine health and disease. Eur J Endocrinol 2014;171(3):R101-110.
8. Saccone D, Asani F, Bornman L. Regulation of the vitamin D receptor gene by environment, genetics and epigenetics. Gene 2015;561(2):171-180.
9. Miettinen ME, Smart MC, Kinnunen L, Mathews C, Harjutsalo V, Surcel HM, et al. Maternal VDR variants rather than 25-hydroxyvitamin D concentration during early pregnancy are associated with type 1 diabetes in the offspring. Diabetologia 2015;58(10):2278-2283.
10. Wang G, Zhang Q, Xu N, Xu K, Wang J, He W, et al. Associations between two polymorphisms (FokI and BsmI) of vitamin D receptor gene and type 1 diabetes mellitus in Asian population: a meta-analysis. PLoS One 2014;9(3):e89325.
11. Mahon BD, Wittke A, Weaver V, Cantorna MT. The targets of vitamin D depend on the differentiation and activation status of CD4 positive T cells. J Cell Biochem 2003;89(5):922-932.
12. Sahin OA, Goksen D, Ozpinar A, Serdar M, Onay H. Association of vitamin D receptor polymorphisms and type 1 diabetes susceptibility in children: a meta-analysis. Endocr Connect 2017;6(3):159-171.
13. Kamel MM, Fouad SA, Salaheldin O, El-Razek Ael R, El-Fatah AI. Impact of vitamin D receptor gene polymorphisms in pathogenesis of Type-1 diabetes mellitus. Int J Clin Exp Med 2014;7(12):5505-5510.
14. Stakisaitis D, Lesauskaite V, Girdauskaite M, Janulionis E,

Ulys A, Benetis R. Investigation of Vitamin D-Binding Protein Polymorphism Impact on Coronary Artery Disease and Relationship with Longevity: Own Data and a Review. Int J Endocrinol 2016;2016:8347379.
15. Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, Nalls M, et al. Vitamin D-binding protein and vitamin D status of black Americans and white Americans. N Engl J Med 2013;369(21):1991-2000.
16. Takiishi T, Gysemans C, Bouillon R, Mathieu C. Vitamin D and diabetes. Endocrinol Metab Clin North Am 2010;39(2):419-446, table of contents.
17. Setty-Shah N, Maranda L, Nwosu BU. Increased risk for vitamin d deficiency in obese children with both celiac disease and type 1 diabetes. Gastroenterol Res Pract 2014;2014:561351.
18. Abd-Allah SH, Pasha HF, Hagrass HA, Alghobashy AA. Vitamin D status and vitamin D receptor gene polymorphisms and susceptibility to type 1 diabetes in Egyptian children. Gene 2014;536(2):430-434.
19. Reis JP, Michos ED, Selvin E, Pankow JS, Lutsey PL. Race, vitamin D-binding protein gene polymorphisms, 25-hydroxyvitamin D, and incident diabetes: the Atherosclerosis Risk in Communities (ARIC) Study. Am J Clin Nutr 2015;101(6):1232-1240.
20. Blanton D, Han Z, Bierschenk L, Linga-Reddy MV, Wang H, Clare-Salzler, M, et al. Reduced serum vitamin D-binding protein levels are associated with type 1 diabetes. Diabetes 2011;60(10):2566-2570.
21. Alam U, Amjad Y, Chan AW, Asghar O, Petropoulos IN, Malik RA. Vitamin D Deficiency Is Not Associated with Diabetic Retinopathy or Maculopathy. J Diabetes Res 2016;2016:6156217.
22. Cagliero E, Levina EV, Nathan DM. Immediate feedback of HbA1c levels improves glycemic control in type 1 and insulin-treated type 2 diabetic patients. Diabetes Care 1999;22(11):1785-1789.
23. Kesavadev J, Sadikot S, Wangnoo S, Kannampilly J, Saboo B, Aravind SR, et al. Consensus guidelines for glycemic monitoring in type 1/type 2 & GDM. Diabetes Metab Syndr 2014;8(3):187-195.
24. Hafez M, Hassan M, Musa N, Abdel Atty S, Azim SA. Vitamin D status in Egyptian children with type 1 diabetes and the role of vitamin D replacement in glycemic control. J Pediatr Endocrinol Metab 2017;30(4):389-394.
25. Taverna MJ, Selam JL, Slama G. Association between a protein polymorphism in the start codon of the vitamin D receptor gene and severe diabetic retinopathy in C-peptide-negative type 1 diabetes. J Clin Endocrinol Metab 2005;90(8):4803-4808.
26. Joergensen C, Tarnow L, Goetze JP, Rossing P. Vitamin D analogue therapy, cardiovascular risk and kidney function in people with Type 1 diabetes mellitus and diabetic nephropathy: a randomized trial. Diabet Med 2015;32(3):374-381.
27. Kaur H, Donaghue KC, Chan AK, Benitez-Aguirre P, Hing S, Lloyd M, et al. Vitamin D deficiency is associated with retinopathy in children and adolescents with type 1 diabetes. Diabetes Care 2011;34(6):1400-1402.
28. Chun RF. New perspectives on the vitamin D binding protein. Cell Biochem Funct 2012;30(6):445-456.
29. Jemielita TO, Leonard MB, Baker J, Sayed S, Zemel BS, Shults J, et al. Association of 25-hydroxyvitamin D with areal and volumetric measures of bone mineral density and parathyroid hormone: impact of vitamin D-binding protein and its assays. Osteoporos Int 2016;27(2):617-626.
30. Perros P, McCrimmon RJ, Shaw G, Frier BM. Frequency of thyroid dysfunction in diabetic patients: value of annual screening. Diabet Med 1995;12(7):622-627.
31. He L, Wang M. Association of vitamin d receptor-a gene polymorphisms with coronary heart disease in Han Chinese. Int J Clin Exp Med 2015;8(4):6224-6229.