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The Effect of Vitamin D Pathway Genes on Asthma Susceptibility, Asthma Control and Vitamin D Levels in Turkish Asthmatic Children

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ABSTRACT The aim of this study was to investigate the associations between the vitamin D (vitD) pathway genes and asthma susceptibility, asthma control and serum vitD levels. Thirty asthmatic children and 30 non-asthmatic (controls) children were genotyped for 9 single nucleotide polymorphisms (SNPs) of vitD pathway genes. These genes were VitD receptor (VDR), 25-hydroxylase (CYP2R1 and CYP27A1), 1-alpha hydroxylase (CYP27B1), 24-hydroxylase (CYP24A1) and VDBP (GC). Genotype and allele frequencies were compared between the groups and their associations with asthma control test (ACT) score and VitD level were investigated. The F allele of VDR1 (FokI) SNP carriers were 2.97 times more likely to develop asthma than those carrying the f allele. Ff genotype of VDR FokI SNP was associated with low ACT score compared to the FF genotype after adjustment. No association between the genotypes and alleles and the level of vitD was found. In conclusion, VDR fokI polymorphism was found to be associated with asthma susceptibility and asthma control in Turkish children.

INTRODUCTION

Asthma is an inflammatory airway disease, of which pathogenesis involves inflammation and airway obstruction. Asthma affects nearly one-eighteen percent of the population worldwide (Global Strategy for Asthma Management and Prevention 2017).

Vitamin D (vitD) is a vitamin, which has effects on the bones and calcium metabolism. About ninety percent of vitD is produced in the skin through the effect of sunlight, and ten percent of this can be maintained by diet. Ultraviolet B light of 290-315 nm wave length affects the skin and converts 7-dehydrocholesterol to vitamin D3, and 25 hydroxylase converts vitD to 25 hydroxy vitD (25(OH)vitD) in the liver (Dusso et al. 2005). A total number of 25(OH) vitD is converted to 1.25 dihydroxy vitD. This is the active form of vitD, and it is mainly produced in the kidneys. Studies have shown that 1 alpha hydroxylase is not only found in the kidneys, but also in other tissues, like the lung, monocytes, and the breast tissue (Hewison et al. 2004). Ninety-nine percent of vitD is transported to the target tissues by proteins, especially by vitamin D binding protein (VDBP) (Chun 2012). VitD binds to vitamin D receptor (VDR), and changes the transcriptions of many genes for biological effects (Wang et al. 2005).

With the identification of VDR in tissues outside the bones, extra-skeletal effects of vitD has been recognized, and research has been undertaken in these pathways. The antiviral, and immunological effects of vitD, and its facilitating effect on the response to steroids as well as its effects on remodeling have demonstrated the association of vitD with asthma cases (Jartti et al. 2010; Sypniewska et al. 2017). The results of the studies on the effects of vitD status on asthma are controversial. While some studies have

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shown that higher 25(OH) vitD levels were associated with better asthma control (Nasiri Kalmarzi et al. 2016; Beyhan-Sagmen et al. 2017), other studies reported no significant associations (Checkley et al. 2015; Kavitha et al. 2017).

Both genetic and environmental factors play a role in asthma pathogenesis. Some genes affect the susceptibility, while some other genes affect the control of asthma (Ullemar et al. 2016).

Some polymorphisms have already been identified in the vitD pathway; like the VDR, vitD 25-hydroxylase (CYP2R1 and CYP27A1), 1 alpha hydroxylase (CYP27B1), 24 hydroxylase (CYP 24A1) and VDBP (GC) (Bosse et al. 2009). With the demonstration of the association between vitD and asthma, there have been studies investigating the role of the single nucleotide polymorphisms (SNP) of genes in the vitD pathway on the development or severity of asthma by either indirectly affecting the levels of vitD or by changing the functioning of vitD. Although, these studies mainly focused on VDR genes' SNPs (Fang et al. 2009; Iordanidou et al. 2014; Einisman et al. 2015; Despotovic et al. 2017), few other studies investigated the association of asthma and the SNPs of VitD pathway genes other than the VDR genes (Aledie et al. 2014; Tizaoui et al. 2014). These studies have conflicting results. Although, there are studies that reported that SNPs in the vitD pathway may be vulnerable as well as protective factor for asthma (Poon et al. 2004; Ismail et al. 2013) and these genes affect the severity of asthma (Ismail et al. 2013; Einisman et al. 2015). There are also studies that demonstrated no such association (Fang et al. 2009; Li et al. 2011; Aledie et al. 2014). Other studies reported that some of these SNPs may affect the levels of vitD (Papadopoulou et al. 2015), although, some researches have not replicated these findings (Li et al. 2011).

Objectives

The current study aimed to investigate the effect of vitD pathway gene polymorphisms (VDR, CYP2R1, CYP27A1, CYP27B1, CYP24A1 and GC) on asthma susceptibility, asthma control test score, and vitD levels in 7- to 17-year-old asthmatic Turkish children.

METHODOLOGY

Thirty asthmatic patients aged 7 to 17 years, who were admitted to Mersin University, School of Medicine, Pediatric Allergy and Clinic Immunology Department between November 2011 and February 2012 were included. Thirty sex and age matched healthy controls were recruited. All asthmatic patients were diagnosed according to the Global Initiative for Asthma (GINA) guideline (Global Strategy for Asthma Management and Prevention 2017). Patients who were only sensitized to house dust mites, and who had intermittent or mild persistent asthma for the last 3 months were recruited. Patients with other chronic lung diseases, with diseases that might affect the vitD levels (liver or kidney disease, malabsorption), and who use vitD containing drugs or drugs affecting the vitD level (systemic steroids, antiepileptic drugs) were excluded. The healthy control group consisted of children who were presented to other departments, and they were selected based on the following criteria: 1. No personal / family history of wheezing, asthma, allergic rhinitisor eczema; 2. No history of low birth weight, premature birth, neonatal mechanical ventilation, or broncho pulmonary dysplasia; 3. No passive smoking in the house; 4. No upper respiratory tract infection in the last two weeks; 5. Normal physical findings; 6. No facial/oral abnormality; 7. No obesity. Written and oral consent from the patients, and parents of each patient were obtained, and the study was approved by the local ethics committee.

Asthma control test (ACT) scores were recorded based on the previous month's asthmatic status. The participants were grouped according to the cut-off score of 20 on the ACT. The patients who have the cut off score of 19 or less are defined as poorly controlled asthmatic group and 20 or more are defined as controlled asthmatic group.

Blood samples were taken for vitD levels and genetic analysis after eight hours of fasting. High performance liquid chromatography system tandem mass spectrometry (HPLC, Chromsystems-Agillent 1100 device) was used to analyse the serum 25(OH) vitD levels of asthmatic patients. Serum 25(OH)vitD levels $\leq$ 20 ng/ml were considered as vitD deficiency, levels between 20 and 30 ng/ml were considered as vitD insufficiency, and levels $\geq$ 30 ng/ml were considered as sufficient (Vieth 2011). A comprehensive questionnaire about demographic data, medications taken for asthma, and factors affecting vitD level (daily sun exposure and the dietary vitD amount per day) was completed. The duration of daily exposure to the sun was grouped as

less than 1 hour per day, 1-3 hours per day, and more than 3 hours per day on most days in the previous month. The parents kept food logs, and the amount of dietary vitD was based only on food intake. VitD content of nutrients have been defined in literature (Ovesen et al. 2003). Body mass index (BMI) was calculated as weight in kilograms divided by the height in squared meters, and recorded at three months interval.

Genetic analyses were conducted at the Medical Biology and Genetics Department of Mersin University, Faculty of Medicine. DNA isolation of peripheral blood in the tubes containing EDTA was performed by kit method. (*Nucleospin Genomic Dna From Blood Macherey-Nagel Germany*). In order to identify the gene polymorphisms of *VDR, GC, CYP2R1, CYP27B1, CYP27A1, CYP24A1,* amplification by polymerase chain reaction was used (Taq DNA Polymerase (Fermentas, EP0402)). Primary sequences used for amplification of different polymorphisms are listed in Table 1. Appropriate restriction enzymes were used to digest the amplified fragments by the restriction fragment length polymorphism (RFLP) process according to the manufacturer's instructions. The restriction enzymes were; *FokI* for VDR (VDR1) (Fermentas, #FD2144), *BsmI* for VDR (VDR2) (Fermentas, #ER0961), *TaqI* for VDR (VDR3) (Fermentas, #ER0671), *ApaI* for VDR (VDR4) (Fermentas, #ER1411), *HaeIII* for GC (Fermentas, #ER0151), *BseGI* for CYP2R1 (Fermentas, #ER0878), PfeI for CYP27B1 (Fermentas, #ER1781), *BseGI* for CYP27A1 (Fermentas, #ER0871), *Bgl II* for CYP24A1 (Fermentas, #ER0081) (Table 1).

After restriction, electrophoresis process was performed and samples were genotyped by an imaging device.

Table 1: Different loci selected within gene sequences, their corresponding primers, PCR and genotype sizes and restriction processes

Gene locus	*Primers*	*Size of PCR amplicon*	*Size of genotype*	*Restriction enzyme, restriction process*
VDR 1	F: 5'-AGC TGG CCT GGC ACT GAC TCT GGC TCT-3'	267 bp	FF:267 bp	*FokI*,
*rs*2228570	R: 5'-ATG GAA ACA CCT TGC TTC TTC TCC CTC-3'		ff:202, 65 bp	37 C° 40 minutes
VDR 2	F: 5'-AAC TTG CAT GAG GAGGAG CAT GTC-3'	801 bp	BB:801 bp	*BsmI*,
rs1544410	R: 5'-GGA GAG GAG CCT GTG TCC CAT TTG-3'		bb:477, 318 bp	37C° 1 night
VDR 3	F: 5'-GGG ACG CTG AGG GAT GGA CAG AGC-3'	714 bp	TT:514, 202 bp	*TaqI*,
*rs*731236	R: 5'-GGA AAG GGG TTA GGT TGG ACA GGA-3'		tt:237, 169, 81 bp65 C°	16 hours
VDR 4	F: 5'-GAC GCT GAG GGA TGG-3'	421 bp	AA: 421 bp	*ApaI*,
*rs*7975232	R: 5'-GTC GGC TAG CTT CTG GAT-3'		aa: 232, 168 bp	37 C° 1 night
GC	F: 5'-AAA TAA TGA GCA AAT GAA AGA AGA C-3'	483 bp	HH:483 bp	*HaeIII*,
*rs*4588	R: 5'-CAA TAA CAG CAA AGA AAT GAG TAG A-3'		hh: 297, 186 bp	37 C° 1 night
CYP2RI	F: 5'-GCC ATA AGT CCA ACC AGG AA-3'	303 bp	FF=303 bp	*FokI*,
rs12794714	R: 5'-GGA AGC TTT GGA GAG CTG AA-3'		ff=173, 130 bp	37 C°
CYP27B1	F: 5'-TTC AAT TCC AGA ACT TCA GAG C-3'	300 bp	TT: 300 bp	*TfiI*,
*rs*10877012	R: 5'-AAC ATA GTC GAA CTG TCT CTA C-3'		tt: 150, 100, 50 bp	37 C° 1 night
CYP27A1	F: 5'-ACC TTC GTC AGA TCC ATC GGG TTA-3'	261bp	BB:261bp	*BseGI*,
*rs*4674338	R: 5'-ATG ATC TCC AAG GAC CAA GAG CCA-3'		bb:153, 108 bp	55C° 1 night
CYP24A1	F: 5'-TGG TTG CAT AAC ACA CAA ACC TA-3'	311 bp	BB: 311bp	*BglII*,
*rs*912505	R: 5'-CTG AAA GCC AGT AAC AAT GGT-3'		bb: 210, 101 bp	37 C° 1 night

Statistical Analysis

SPSS for Windows 11.5 was used for the analyses. For comparison of the patients and the control groups in terms of age, the Student's t-test was used. Pearson's chi-square test and Fisher's exact test were used for categorical variables. Odds ratios and ninety-five percent confidence intervals were calculated to demonstrate the association of gene polymorphisms with asthma susceptibility and vitD levels in terms of genotype and allele frequencies. Multivariate regression models were used for the analysis of the associations of SNPs, and the ACT scores and vitD levels. Values of $p < 0.05$ were considered as significant.

RESULTS

The mean age of asthmatic patients was 11.74 ± 2, 4 years and the mean age of the control group was 11.41±2.27 years. Ten of the asthmatic patients (33.3%) and 17 (56.6%) of the controls were girls. The cases and control group participants did not differ from each other in age (p value: 0.38) and sex distribution (p value: 0.07). Fifteen (50%) of the asthmatic patients were under a low dose inhaled corticosteroid (ICS) therapy.

Asthmatic patients and healthy controls were compared according to the frequencies of SNPs of VDR gene (four different restriction sites), VDBP gene (GC), 25-hydroxylase genes (CYP27A1 and CYP2R1), 1-alpha-hydroxilase gene (CYP27B1) and 24-hydroxilase gene (CYP24A1). Genotype frequencies were not significantly different between the groups. Genotype frequencies of asthmatics and healthy controls are shown in Table 2.

The F allele of VDR1 (FokI) gene polymorphism was more frequent in the asthmatic group

Table 2: The distribution of genotypes between the asthmatic children and healthy controls

	Healthy controls (n:30)	*Asthmatic children (n:30)*	*p value*	*OR (95% CI)*
VDR 1				
Ff	6 (20.0%)	0 (0%)	-	1.000
Ff	12 (40.0%)	11 (36.7%)	0.791	-
FF	12 (40.0%)	19 (63.3%)	0.071	-
VDR 2				
bb	12 (40.0%)	16 (53.3%)	-	1.000
Bb	13 (43.3%)	12 (40.0%)	0.506	0.692 (0.234-2.048)
BB	5 (16.7%)	2 (6.7%)	0.190	0.300 (0.049-1.820)
VDR 3				
T t	5 (16.7%)	3 (10.0%)	-	1.000
T t	10 (33.3%)	9 (30.0%)	0.638	1.500 (0.276-8.138)
T T	15 (50.0%)	18 (60.0%)	0.392	2.000 (0.409-9.777)
VDR 4				
Aa	23 (76.7%)	25 (83.3%)	-	1.000
AA	7 (23.3%)	5 (16.7%)	0.519	0.657 (0.183-2.363)
GC				
hh	9 (31.0%)	9 (32.1%)	-	1.000
Hh	16 (55.2%)	16 (57.1%)	1.000	1.000 (0.315-3.174)
HH	4 (13.8%)	3 (10.7%)	0.749	0.750 (0.129-4.356)
CYP2R1				
bb	12 (41.4%)	12 (40.0%)	-	1.000
Bb	12 (41.4%)	9 (30.0%)	0.632	0.750 (0.231-2.435)
BB	5 (17.2%)	9 (30.0%)	0.395	1.800 (0.464-6.976)
CYP27B1				
pp	6 (20.7%)	0 (0.0%)	-	1.000
Pp	16 (55.2%)	19 (76.0%)	0.110	-
PP	7 (24.1%)	6 (24.0%)	0.991	-
CYP27A1				
bb	27 (93.1%)	26 (100.0%)	-	1.000
BB	2 (6.9%)	0 (0.0%)	0.492	-
CYP24A1				
bb	3 (10.0%)	3 (10.3%)	-	1.000
Bb	19 (63.3%)	17 (58.6%)	0.900	0.895 (0.159-5.041)
BB	8 (26.7%)	9 (31.0%)	0.901	1.125 (0.175-7.243)

than in the healthy control group. Children carrying the F allele of VDR1 (FokI) gene were 2.97 times more likely to develop asthma [OR = 2.97, 95% CI (1,291-6,883), p = 0,009] than those carrying the f allele. Allele frequencies of the genes other than VDR1 were not significantly different between the asthmatic and the healthy control groups (Table 3).

All of the participants were divided into two groups according to the vitamin D level. The genotype and allele frequencies of the vitD sufficient (vitD level ≥30 ng/mL) and the vitD insufficient/deficient (vit D level <30 ng/mL) groups were not significantly different (Table 4, Table 5).

The association of vitD pathway gene polymorphisms and ACT score (<20 or ≥20 points) in asthmatic patients was evaluated by a multivariate logistic regression model which was adjusted for age, gender, ICS use, and BMI. Since the genotype of all the asthmatic patients was bb, CYP27A1 was not included in the analysis. According to this model, the Ff genotype of VDR FokI polymorphism was associated with low ACT score (OR = 0.05, 95% CI (0.003-0.864), p = 0.039) compared with the FF genotype after adjustment for covariates. There were no association between other genes and ACT score (Table 6).

The predictive role of genotypes on the levels of vitD (sufficient vs. insufficient/deficient) was also investigated with a regression model, where exposure to sunlight, age, BMI, and the amount of vitD in the diet were controlled. No significant association between the genotypes and the level of vitD was found (data not shown).

DISCUSSION

Previous studies reported that in asthmatic children, there is an association between vitD deficiency and asthma susceptibility or asthma control (Dogru et al. 2014; Beyhan-Sagmen et al. 2017; Hollams et al. 2017).

With the expansion of studies focusing on the genetic basis of asthma, various studies on the relationship between vitD pathway gene polymorphisms and asthma have been undertaken in different populations. In this study, the results indicated that the F allele of VDR FokI polymorphism was significantly more frequent

Table 3: The distribution of allele frequencies in the asthmatic children and healthy controls

Alleles	*Healthy controls (n:30)*	*Asthmatic children (n:30)*	*p value*	*OR (95% CI)*
VDR 1				
f	24 (40.0%)	11 (18.3%)	-	1.000
F	36 (60.0%)	49 (81.7%)	0.009	2.970 (1.291-6.883)
VDR 2				
b	37 (61.7%)	44 (73.3%)	-	1.000
B	23 (38.3%)	16 (26.7%)	0.172	0.585 (0.270-1.268)
VDR 3				
t	20 (33.3%)	15 (25.0%)	-	1.000
T	40 (66.7%)	45 (75.0%)	0.315	1.500 (0.678-3.317)
VDR 4				
a	23 (38.3%)	25 (41.7%)	-	1.000
A	37 (61.7%)	35 (58.3%)	0.709	0.870 (0.419-1.808)
GC				
h	34 (58.6%)	34 (60.7%)	-	1.000
H	24 (41.4%)	22 (39.3%)	0.820	0.917 (0.434-1.938)
CYP2R1				
b	36 (62.1%)	33 (55.0%)	-	1.000
B	22 (37.9%)	27 (45.0%)	0.436	1,339 (0.642-2.792)
CYP27B1				
p	28 (48.3%)	19 (38.0%)	-	1.000
P	30 (51.7%)	31 (62.0%)	0.283	1.523 (0.706-3.286)
CYP27A1				
b	56 (96.6%)	52 (100.0%)	-	1.000
B	2 (3.4%)	0 (0.0%)	0.497	-
CYP24A1				
B	25 (41.7%)	23 (39.7%)	-	1.000
B	35 (58.3%)	35 (60.3%)	0.824	1.087 (0.521-2.267)

Table 4: The genotype frequencies of the vitD sufficient and the vitD insufficient/deficient groups

Genotypes	*vitD <30 (n:24)*	*vitD ≥30 (n:6)*	*p value*	*OR (95% CI)*
VDR 1				
Ff	9 (37.5%)	2 (33.3%)	-	1.000
FF	15 (62.5%)	4 (66.7%)	0.850	1.200 (0.182-7.926)
VDR 2				
bb	12 (50%)	4 (66.7%)	-	1.000
Bb	11 (45.8%)	1 (16.7%)	0.302	0.108 (0.202-4.155)
BB	1 (4.2%)	1 (16.7%)	0.440	1.190 (0.068-16.388)
VDR 3				
t t	2 (8.3%)	1 (16.7%)	-	1.000
T t	7 (29.2%)	2 (33.3%)	0.784	1.020 (0.060-11.917)
T T	15 (62.5%)	3 (50%)	0.597	1.540 (0.559-16.837)
VDR 4				
Aa	20 (83.3%)	5 (83.3%)	-	1.000
AA	4 (16.7%)	1 (16.7%)	0.876	1.000 (0.091-11.028)
GC				
hh	6 (25%)	3 (50%)	-	1.000
Hh	14 (58.3%)	2 (33.3%)	0.329	1.854 (0.743-9.113)
HH	3 (12.5%)	-	0.509	-
CYP2R1				
bb	9 (37.5%)	3 (50%)	-	1.000
Bb	7 (29.2%)	2 (33.3%)	0.719	0.557 (0.159-3.903)
BB	8 (33.3%)	1 (16.7%)	0.262	0.478 (0.290-4.358)
CYP27B1				
P p	16 (72.7%)	3 (100%)	-	1.000
P P	6 (27.3%)	-	0.299	-
CYP27A1				
bb	22 (100.0%)	4 (100.0%)	-	1.000
CYP24A1				
bb	2 (8.3%)	1 (16.7%)	-	1.000
Bb	13 (54.2%)	4 (66.7%)	0.763	1.013 (0.540-4.553)
BB	8 (33.3%)	1 (16.7%)	0.234	1.232 (0.807-3.677)

Table 5: The allele frequencies of the vitD sufficient and the vitD insufficient/deficient groups

SNPs	*VitD level <30*	*VitD level ≥ 30*	*p-value*	*OR (95% CI)*
VDR 1				
F	40 (83.3%)	9 (75%)		1
f	8 (16.7%)	3 (25%)	0.505	0.600 (0.132-2.719)
VDR 2				
B	12 (25%)	4 (33.3%)		1
b	36 (75%)	8 (66.7%)	0.559	1.500 (0.382-5.883)
VDR 3				
T	37 (77.1%)	8 (66.7%)		1
T	11 (22.9%)	4 (33.3%)	0.456	0.595 (0.150-2.354)
VDR 4				
A	28 (58.3%)	7 (58.3%)		1
A	20 (41.7%)	5 (41.7%)	1	1 (0.277-3.608)
GC				
H	19 (41.3%)	3 (30%)		1
h	27 (58.7%)	7 (70%)	0.507	0.609 (0.139-2.660)
CYP2R1				
B	23 (47.9%)	4 (33.3%)		1
B	25 (52.1%)	8 (66.7%)	0.364	0.543 (0.144-2.049)
CYP27B1				
P	26 (61.9%)	5 (62.5%)		1
P	16 (38.1%)	3 (37.5%)	0.975	1.260 (0.215-4.886)
CYP24A1				
B	29 (60,4%)	6 (60%)		1
B	19 (39.6%)	4 (40%)	0.98	0.983 (0.245-3.950)

Table 6: The association of genotypes and ACT score groups in asthmatic patients

SNP	*B*	*p*	*OR*	*95% CI*
VDR1				
Ff vs FF	-3.002	0.039	0.05	0.003-0.864
VDR2				
Bb vs BB	-0.742	0.661	0.476	0.017-13.208
bb vs BB	-0.605	0.741	0.546	0.015-19.656
VDR3				
Tt vs TT	-0.438	0.692	0.645	0.074-5.632
tt vs TT	-0.581	0.722	0.56	0.023-13.675
VDR4				
Aa vs AA	-0.838	0.514	0.433	0.035-5.361
GC				
Hh vs HH	19.953	0.918	8.759	0.785-97.754
hh vs HH	20.369	0.854	7.12	0.914-61.4
Cyp27B1				
Pp vs PP	0.158	0.928	1.172	0.039-35.547
Cyp24A1				
Bb vs BB	1.496	0.278	4.463	0.300-66.444
bb vs BB	23.574	0.904	3.54	0.433-28.971
Cyp2R1				
Bb vs BB	1.769	0.236	5.863	0.315-109.032
bb vs BB	2.056	0.124	7.815	0.570-107.168

Multivariate models were adjusted by age, gender, BMI, ICS use

in asthmatic children than the healthy controls and the Ff genotype of VDR FokI polymorphism is associated with low ACT score compared to the FF genotype after adjustment for covariates.

The genetic variations (SNPs) of the VDR gene may influence the expression or the function of VDR, and therefore it may alter the activity of vitD. VDR gene polymorphisms are the most frequently researched genes in terms of its association with asthma (Iordanidou et al. 2014; Tizaoui et al. 2014; Despotovic et al. 2017). So far, four SNPs of the VDR gene (FokI, BsmI, ApaI and TaqI) have been properly investigated by genetic association studies. In these studies, the effects of the VDR gene polymorphisms on the susceptibility of asthma as well as their effects on the asthma control and vitD level were evaluated (Iordanidou et al. 2014; Einisman et al. 2015).

Two studies from Tunisia and Egypt showed that the VDR FokI SNP FF genotype and the F allele were over represented in asthmatics than the controls, as was the case in this study (Li et al. 2011; Ismail et al. 2013). On the other hand, another two studies reported the under representation of FokI FF genotype and the F allele (Despotovic et al. 2017) and Apa I SNP AA genotype in asthmatic patients (Saadi et al. 2009). Also, there are studies that reported no associations with VDR gene polymorphisms and asthma (Fang et al. 2009; Iordanidou et al. 2014). According to the results of two recent meta-analysis, one reported an association of ApaI, FokI, BsmI and another reported an association with ApaI, FokI and TaqI with asthma susceptibility (Han et al. 2016; Zhao et al. 2017).

There are also some studies in which the associations of the VDR SNPs and asthma control were investigated. In one of these studies, it was reported that the ACT scores were higher in asthmatics carrying the ApaI aa genotype (Papadopoulou et al. 2015). Yet, the other found a relationship of decreased lung function with the VDR fokI FF genotype (Ismail et al. 2013), while in another study, no association between the VDR SNPs and asthma control was documented (Li et al. 2011). Since asthma control is affected by many factors other than genes, in this study, the associations of the vitD pathway gene polymorphisms and ACT scores after an adjustment for age, gender, ICS use and BMI were analysed. The Ff genotype of VDR FokI polymorphism was associated with worse ACT score compared to FF genotype after adjustment for covariates. Although the odds ratio was low, this result might have clinical implications such that the genotype profile may give a change to predict the prognosis of asthmatic patients at early ages.

One of the reasons for the association of asthma and the SNPs of vitD pathway genes

may be through the effect of SNPs on the vitD levels. Some studies found an association of the TaqI tt genotype with higher (Papadopoulou et al. 2015), and the FokI FF genotype with lower vitD levels (Ismail et al. 2013). However, from previous reports and similar to this study's results, SNPs and vitD levels showed no association before and after adjustments for covariates (Maalmi et al. 2013).

VDBP (GC globulin) is a protein that transports the vitD metabolites. GC is a gene located at 4q11–13 (Speeckaert et al. 2006). VDBP has two most common genetic variants—D432E (rs7041) and T436K (rs4588), which encode the proteins Gc1F/Gc1S and Gc2 (Kamboh and Ferrell 1986). There are few studies in the literature evaluating the relationship of GC SNPs and asthma. One of them found an association between rs4588 and rs7041 variants with serum vitamin D and/or VDBP levels. Also, the researchers suggested that the Gc1S allele confers a protective effect with respect to the development of asthma (Aledie et al. 2014). Another study found that GC 2/2 genotype confers a significant risk for developing asthma, while no associations with genotypes and vitD levels were reported (Li et al. 2011). To the best of our knowledge, there are only two studies in the literature that discuss the effect of VDBP SNPs to asthma control. One of these studies reported that the rs2228570 GG genotype was related to more severe asthma (Ismail et al. 2013), whereas, the other study reported no association of 6 different SNPs (which also contained the SNP that was evaluated in our study) with asthma risk or severity (Leung et al. 2015). In this study, no association was found with the VDBP genetic variants and asthma control and vitD levels as some other studies in the literature. One possible explanation may be the small sample size, and the evaluation of only one SNP, ignoring diplotypes in this study.

CYP2R1 and CYP27A1 encode the 25-hydroxylase enzyme. Only one of the limited number of previous studies has reported an over-expression of the TT haplotype of the CYP27A1 gene SNPs in asthmatic patients (Leung et al. 2015). Also, two studies found an association between lung function tests and CYP2R1 SNPs (Pillai et al. 2011; Leung et al. 2015) Only a few number of studies found no association between asthma susceptibility, asthma control and vitD levels as this study (Bosse et al. 2009; Li et al. 2011).

The active form of vitamin D is 1á, $25(OH)_2$vitD, and it is converted by 1á-hydroxylase (encoded by the CYP27B1 gene) from 25(OH)vitD, and is inactivated by 24-hydroxylase (encoded by the CYP24A1 gene). A study found an association between asthma and atopy in CYP24A1 genetic variants (Bosse et al. 2009), two other studies failed to detect any association (Wjst et al. 2006; Bosse et al. 2009). Additionally, in the literature, the SNP of CYP24A1 rs2248137 was associated with better lung function tests (Pillai et al. 2011). In this study, no significant associations were found between these genes and the evaluations undertaken.

As vitD levels may be affected by not only genes but also sunlight exposure, dietary vitD level, BMI, age, these variables were entered as covariates, and the analyses were repeated. In this study, no association was found between the genes and vitD levels after the effect of the confounders was controlled.

This is the first study that shows the associations among vitD pathway genetic variants and asthma susceptibility, asthma control and vitD levels in Turkish asthmatic children. The major reason for the conflicting results from different studies may be ethnic, geographic and differences in living habit between the populations. Another reason for the conflicting results may be the different asthma definitions in different studies. Since the patients were suffering from intermittent or mild persistent asthma, this may also have influenced the relationship between the SNPs and the ACT scores. Furthermore, there are several other genes and factors that may affect asthma susceptibility, asthma control and vitD levels. Also, gene-gene and gene environment interactions may not be totally excluded.

The limitations of this study are its small sample size, limited SNPs of different genes for analysis, not taking into account the two gene models, and no knowledge of previous study in a Turkish population. One of the strengths of this study is that the effects of the covariates were taken into account while evaluating the effect of asthma control and vitD levels. Although, there are some studies about vitD pathway genes and asthma control that took into account the covariates (Pillai et al. 2011; Iordanadou et al. 2014; Leung et al. 2015), to the best of the researchers' knowledge, there are no other

reports focusing on the association between vitD pathway gene polymorphisms and vitD status in asthmatic children following this study design. Other strengths of this study are the investigation of almost all genes in the pathway, and the objective asthma diagnosis by a physician.

CONCLUSION

The results of the present study have demonstrated that there is an association between genetic variants of the VDR gene and asthma in Turkish children. In addition to the known asthma risk genes, understanding the role of vitD pathway genes in asthma pathogenesis might inform better asthma control and treatment.

RECOMMENDATIONS

Larger population based studies in Turkish children are still needed in order to validate the associations.

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