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Genetic variants in vitamin D pathway in Egyptian asthmatic children: A pilot study

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ABSTRACT

Objectives: Asthma is a genetically heterogeneous disease. Genetic variants in vitamin D pathway have been reported to be involved with asthma risk. The study aimed to test whether vitamin D binding protein (VDBP or GC-group component) and vitamin D receptor (VDR) gene polymorphisms were associated with asthma characteristics as well as vitamin D level in Egyptian children.

Design and methods: The study included 51 asthmatic children and 33 healthy controls of matched sex and age. All participants were genotyped for two SNPs; GC (rs2282679) and VDR (rs2228570) using Taq-Man allele discrimination assays.

Results: Genotype distribution of GC and VDR showed a significant association with asthma ($P = 0.02$, $P = 0.002$). Children carrying the risk “G” allele for GC SNP are 2.22 times more prone to develop asthma [OR = 2.22, 95% CI (1.18–4.2)] whereas those carrying the risk “F” allele for VDR SNP are nearly twice and half times susceptible for asthma development [OR = 2.68, 95% CI (1.36–5.28)] than healthy individuals. For the GC SNP, homozygous children “GG” exhibited significant difference in pulmonary functions (FEV1, FEV1/FVC), asthma severity and asthma control, IgE and vitamin D levels compared to pooled cases of GT and TT genotypes. For the VDR SNP, no significant association between VDR variants and the tested characteristics except for the pulmonary functions where the FEV1/FVC in asthmatic children with “FF” genotype differ significantly from those carrying “FF” genotype.

Conclusion: GC and VDR variants may be implicated in asthma susceptibility; hence, further larger studies are still needed to extrapolate our findings to the general population.

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1. Introduction

Asthma is a chronic, genetically heterogeneous disease characterized by airways inflammation with recurrent symptoms ranging from mild to debilitating. Due to disease complexity, identification of the causal genes has been regarded as a challenging task. The immune system plays a pivotal role in asthma pathogenesis leading to inflammation, airway hyperresponsiveness, and mucus obstruction [1]. Recently, the vitamin D pathway has been regarded as a contributing factor to the outcome of immune responses. It has been shown that low vitamin D levels are linked to increased asthma severity in children [2] and increased prenatal vitamin D intake may reduce childhood asthma incidence [3]. Vitamin D synthesis, transport and degradation are controlled by several genes where genetic polymorphisms may affect vitamin D

status. Interestingly, some polymorphisms have been detected in genes encoding components of the vitamin D pathway; including the vitamin D binding protein (VDBP, or GC-group component) and the vitamin D receptor (VDR).

Vitamin D synthesis is induced by ultraviolet B radiation which converts 7-dehydrocholesterol (present in the skin) to vitamin D3. Most of the vitamin D, either from dietary source or from skin synthesis, is hydroxylated in the liver to give 25(OH) D. In the kidneys, the 25(OH) D will be further hydroxylated to produce 1,25-dihydroxyvitamin D [1,25(OH) 2D, calcitriol]. Vitamin D and its metabolites are transported in the circulation bound to VDBP [4]. Evidence for the association of GC gene polymorphism with vitamin D levels is continuously increasing [5]. Two genome-wide association studies (GWAS) of 25(OH) D levels in Europeans reported an association with a single nucleotide polymorphism (SNP) in GC (rs2282679) [6,7]. In the circulation, vitamin D metabolites bound to VDBP are less prone to hydroxylation and degradation; hence they have a longer $t_{1/2}$, thus implicating VDBP in stabilizing and maintaining circulating levels of vitamin D [8].

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Identification of VDR in antigen-presenting cells and activated T lymphocytes supports the hypothesis that vitamin D pathway plays a role in immune-related diseases such as asthma [9]. VDR is located on the long arm of chromosome 12, a region that has been linked to asthma and allergy-related phenotypes in genome-wide linkage analyses [10,11]. VDR belongs to a family of ligand-activated transcription factors [12]. Interestingly, VDR polymorphisms have been associated with some inflammatory disorders, however, the exact mechanism of action remains to be speculated. It has been shown that the VDR polymorphism (FokI, rs2228570) could interfere with transcription factors signaling in immune-mediated diseases [13].

Having already collected a cohort of 51 fully phenotyped children with asthma, we chose to conduct a pilot study to investigate the associations of known vitamin D-related SNPs with vitamin D level as well as with asthma characteristics including airflow limitation, and markers representing allergy such as serum total IgE and blood eosinophil count.

2. Subjects and methods

2.1. Subjects

The study included fifty one asthma patients and thirty three controls where all participants were unrelated. Asthma cases were recruited from the Pediatric Chest Clinic, Children's Hospital, Ain Shams University. They were diagnosed according to the clinical manifestations (Cough, wheezing, shortness of breath and exercise intolerance) in agreement with Global Initiative for Asthma (GINA) guidelines [14] and confirmed by spirometry. Dynamic spirometry (Jaeger, Germany) was performed, with measurement of forced expiratory volume in 1 s (FEV1) (% of predicted) and the forced vital capacity (FVC). The ratio FEV1/FVC is a measure of airflow obstruction. These measurements were performed according to the standards of the European Respiratory Society and the American Thoracic Society [15]. The highest values of FEV1 of three forced expiratory manipulations were used. The best FEV1, FVC and FEV1/FVC values were selected for analysis. Information on age, sex, parental consanguinity, family history of allergic disease, duration of illness and treatment modalities were collected via a questionnaire. Thirty three healthy children of matched age and sex were included in the study and served as control group. A written informed consent was obtained from the study participants and their parents. The study was conducted in accordance with ethical procedures and policies approved by the ethical Committee of Faculty of Pharmacy Cairo University, Cairo, Egypt.

The asthmatic patients were further subdivided according to asthma control during the past three months into controlled ($n = 20$; 39.2%), partially-controlled ($n = 14$; 27.5%) and uncontrolled ($n = 17$; 33.3%) [14]. Regarding asthma severity, the patients were classified into mild persistent ($n = 20$; 39.2%), moderate persistent ($n = 15$; 29.4%) and severe persistent ($n = 16$; 31.4%) [14]. The patients were also classified according to the intake of inhaled corticosteroid (ICS) therapy into ICS group ($n = 38$; 74.5%) and non-ICS group ($n = 13$; 25.5%) (Table 1).

2.2. Biochemical measurements

After an overnight fast, venous blood samples were collected from all participants and the separated serum was stored at -20°C for biochemical assessment of vitamin D and IgE levels. Another portion of blood sample was collected on EDTA for eosinophilic count using Coulter counter technique. Quantitative determination of the 25-OH vitamin D in serum samples was performed using Enzyme-Linked Immunosorbent Assay (ELISA),

Table 1
Clinical characteristics of the participants.

Characteristic	Cases ($n = 51$)	Controls ($n = 33$)	P value
Age, yr	8.6 ± 2.7	7.8 ± 2.6	0.2*
Sex, n (%)			
Males	28 (54.9)	18 (54.5)	1.0 [®]
Females	23 (45.1)	15 (45.5)	
Family history, n (%)	29 (56.9)		
Consanguinity, n (%)	33 (64.7)		
Duration of illness, yr	5.8 ± 2.6		
Treatment modality, n (%)			
Inhaled corticosteroids (ICS)	38 (74.5)		
No (ICS)	13 (25.5)		
Eosinophils count, cells/mm ³	405.7 ± 147	161 ± 52.5	0.0001*
IgE, mean $\pm$ SD (IU/mL)	70.9 ± 37.4	17.7 ± 9.3	0.0001*
Vitamin D, mean $\pm$ SD (ng/mL)	25.2 ± 3.4	41.3 ± 4.6	<0.001*
Vit D			
+ve consanguinity ($n = 33$)	25.9 ± 2.9^a	41.3 ± 4.6^b	<0.001*
– ve consanguinity ($n = 18$)	23.9 ± 4.0^a		
Pulmonary functions:			
FEV1 (%predicted)	66.1 ± 6.6		
FVC	94.4 ± 3.1		
FEV1/FVC	69.9 ± 6		
Asthma severity, n (%)			
Mild	20 (39.2)		
Moderate	15 (29.4)		
Severe	16 (31.4)		
Asthma control, n (%)			
Controlled	20 (39.2)		
Partially controlled	14 (27.5)		
Uncontrolled	17 (33.3)		

IgE: immunoglobulin E; FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity.

Data are mean $\pm$ SD for quantitative variables, or number (%) for qualitative ones. Groups having different letter label are statistically significantly different at $p < 0.05$ (Tukey's post hoc test).

* Independent samples *t*-test.

[®] Fisher exact test. $P < 0.05$ is considered significant.

* One way ANOVA.

(Immundiagnostik EIA, Bensheim and Biomedica, Wien/Austria), according to manufacturer's guidelines [16]. All wash steps were performed using an ELISA washer (Robonik ELISA plate washer), whereas the absorbance of all samples were read using the ELISA reader (Biotek ELx800) at 450 nm. A standard curve of the absorbance vs. concentration was plotted using the calibrators. Concentration of vitamin D in the samples was determined directly from the curve. Serum vitamin D levels were categorized as deficient if vitamin D level was <25 ng/ml, and insufficient if it was 25–30 ng/ml. Serum vitamin D level was considered normal or optimal if above 30 ng/ml. Quantitative determination of serum total IgE was performed using ELISA, (RIDASCREEN Total IgE (A0141), R-Biopharm AG, Darmstadt/Germany), according to manufacturer's guidelines. All wash steps were performed using an ELISA washer (Robonik ELISA plate washer), whereas the absorbance of all samples were read using the ELISA reader (Biotek ELx800) at 405 nm. A standard curve of the absorbance unit vs. concentration was generated using the results obtained from the standards provided with the kit. Total IgE values for samples were determined directly from the curve.

2.3. Genotyping

Genomic DNA was isolated from frozen packed white blood cells according to manufacturer's instructions using a commercial kit QIAampDNA Blood Mini Kit (Qiagen, Germany). Genotyping was done using TaqMan allele discrimination assays (Applied Biosystems, Foster City, CA). Both alleles were detected simultaneously using allele-specific oligonucleotides labeled with different fluorophores and genotypes automatically determined

by the ratio of the two fluorophores used from the TaqMan Genotyping Universal PCR Master Mix (Applied Biosystems). Polymerase chain reaction and allele calling were performed on StepOne Realtime PCR (Applied Biosystems).

2.4. Statistical analysis

Data were statistically analyzed by the Statistical Package of Social Science Software program (SPSS), version 21. Data were presented as mean $\pm$ standard deviation for quantitative variables or frequency and percentage for qualitative ones. Comparison between groups was done using independent sample *t*-test for quantitative variables and Pearson's chi square test or Fisher's exact test for qualitative ones. *P* values $\leq$ 0.05 were considered statistically significant.

3. Results

Of the Egyptian asthmatic children ($n = 51$), 54.9% were male, where the mean age $\pm$ SD was 8.6 ± 2.7 years and duration of illness was 5.8 ± 2.6 years. Family history and consanguinity were detected in 56.9% and 64.7%, respectively of cases. Notable asthma-relevant characteristics included serum IgE = 70.9 ± 37.4 IU/mL, eosinophilic count = 405.7 ± 147.5 cells/mm³ and FEV1/FVC = 69.9 ± 6 . The mean vitamin D level for our asthma cohort was 25.2 ± 3.4 ng/mL. The control cohort ($n = 33$) had 54.5% male subjects with mean age (SD) of 7.8 (2.6) years with no significant difference from the asthma cohort. However, a significant difference was detected in serum IgE (17.7 ± 9.3), eosinophilic count (161 ± 52.5) and vitamin D level (41.3 ± 4.6) in comparison to asthmatic children. Regarding consanguinity, no significant difference was found in vitamin D level in asthmatic patients with +ve and -ve consanguinity (25.9 ± 2.9 ; 23.9 ± 4.0 , respectively). Clinical characteristics of the participants are presented in Table 1.

For the GC rs2282679 and VDR rs2228570, the frequency of GG and FF genotypes in the asthmatic group was 41.2% and 56.9%, respectively with a significant difference from the respective control values (24.2%, 36.4%); ($X^2 = 8.2$, $X^2 = 12.6$); ($P = 0.017$, $P = 0.002$). The alleles frequencies were significantly different as evidenced by 67.6% prevalence of "G" allele and 78.4% prevalence of "F" allele in asthmatic patients versus 51.5% and 57.6% in the controls ($X^2 = 5.4$, $X^2 = 7.4$), ($P = 0.02$, $P = 0.006$). Children carrying the "G" allele are 2.22 times more likely to develop asthma [OR = 2.22, 95% CI (1.18–4.2)] whereas those carrying the "F" allele are nearly twice and half times susceptible for asthma development [OR = 2.68, 95% CI (1.36–5.28)] than healthy individuals. It is noteworthy to mention that our asthma cohort does not include children carrying the "ff" genotype. In multiple comparisons for different genotypes distribution, GG in relation to TT and GT in relation to TT in asthmatic and control groups showed significant difference ($P = 0.04$; $P = 0.08$, respectively). Regarding VDR gene, FF versus ff and Ff versus ff revealed significant difference ($P = 0.002$; 0.01, respectively) in cases and controls groups (Table 2).

Relation between genetic variants of GC and VDR genes and asthma characteristics are shown in Table 3. For the GC rs2282679, asthma characteristics did not differ significantly among the three different genotypes. However, pooling of the two genotypes with the wild allele (GT + TT) revealed significant difference in pulmonary functions such as FEV1 (68.8 ± 6) and FEV1/FVC (72.7 ± 5.1) compared to homozygous patients with risk GG allele (62.1 ± 5.3 ; 66 ± 5.1 , respectively), ($P = 0.001$, $P = 0.001$). Regarding asthma severity and asthma control, 61.9% and 71.4% of asthmatic patients with homozygous risk "G" allele suffered from severe asthma symptoms and uncontrolled disease, respectively as compared to only 10% and 6.7% of asthmatic patients with

pooled genotypes (GT + TT) ($P = 0.001$). Regarding multiple comparisons, severe versus mild disease was significantly associated with GG genotypes in comparison to GT + TT genotypes ($P = 0.003$). Moreover, severe versus moderate disease was also significantly associated with GG genotypes compared to GT + TT genotypes ($P = 0.03$). Regarding asthma control, patients with GG genotypes suffered from uncontrolled versus controlled disease, in a significant manner, compared to patients with GT + TT genotypes ($P = 0.001$). In the same line, uncontrolled versus partially controlled disease was significantly associated with patients with GG genotypes in comparison to GT + TT genotypes ($P = 0.001$). By analyzing the distribution of vitamin D and IgE levels in different genotypes, a significant association was revealed in (GT + TT) versus GG asthmatic children (27.2 ± 2.4 ; 57.9 ± 30.4 , respectively) versus (22.4 ± 2.5 ; 89.6 ± 39.1 , respectively) ($P = 0.001$, $P = 0.002$). Within both +ve and -ve consanguinity patients, a significant decrease in vitamin D level was associated with GG genotypes compared to GT+TT genotypes ($P = 0.001$; $P = 0.001$, respectively). For the VDR rs2228570, no significant association of the clinical and laboratory characteristics among the different genotypes except for vitamin D level in -ve consanguinity patients and for the pulmonary functions where the FEV1/FVC in asthmatic children with "Ff" genotype (72 ± 4.8) differ significantly from those carrying "FF" genotype (68.3 ± 6.4) at $P = 0.02$. Within -ve consanguineous patients, decreased vitamin D level was significantly associated with FF genotype compared to Ff genotype ($P = 0.01$).

4. Discussion

Heritability of vitamin D status seems to be considerable, however, the specific genetic determinants of vitamin D levels are only beginning to be identified. To the best of our knowledge, this is the first pilot study using a cohort of Egyptian children with high prevalence of both asthma and hypovitaminosis D in whom we attempted to determine associations with two SNPs (GC rs2282679 and VDR rs2228570) in vitamin D-related genes and asthma characteristics. Recently, lower vitamin D concentrations have been found to be associated with higher risks of asthma exacerbations (e.g., hospitalization and use of anti-inflammatory drugs) and increased levels of serum total IgE and peripheral eosinophil count in Costa Rican children with asthma [17]. In the current study, an elevation in IgE level and eosinophil count, together with a marked decline in lung function (FEV1/FVC) and vitamin D level (in both +ve and -ve consanguineous cases) were detected in asthmatic children. In a cross-sectional study based on the US Third National Health and Nutrition Examination Survey, a dose-dependent association between lower serum 25OHD and reduced pulmonary function, as assessed by FEV1 and FVC, was observed among participants, most of whom did not have asthma [18]. During pregnancy, higher maternal vitamin D intake was inversely related to asthma manifestations (such as wheezing) in children aged from three to five years old, suggesting the timing (prenatal vs. postnatal) of vitamin D administration may have an impact on respiratory disease [3,19]. In steroid-resistant asthma, administration of vitamin D reversed resistance through induction of interleukin 10-secreting T-regulatory cells [20].

The human VDBP-gene maps to the long arm of chromosome 4. The protein product is composed of 458 amino acids where two binding regions have been identified: a vitamin D binding domain between residues 35 and 49 and an actin binding domain between residues 373 and 403 [21]. Analyses of the four GC SNPs (rs7041–rs4588–rs1155563–rs2282679) suggested that the rs4588 and/or rs2282679 may be associated with vitamin D levels [22]. In two GWAS studies [6,7], it has been demonstrated that the GC-rs2282679 was identified as the strongest association signal for

Table 2
Genotype and allele frequencies of GC and VDR genes in all participants.

Genotype	Cases <i>n</i> (%) (<i>n</i> = 51)	Controls <i>n</i> (%) (<i>n</i> = 33)	Multiple comparison <i>P</i> value [Ⓐ]	OR (95% CI)	χ ²	<i>P</i> value
<i>(GC) genotype</i>						
GG	21 (41.2%)	8 (24.2%)			8.2	0.017[Ⓐ]
GT	27 (52.9%)	16 (48.5%)				
TT	3 (5.9%)	9 (27.3%)				
GG/GT	21/27	8/16	0.5 [Ⓐ]			
GG/TT	21/3	8/9	0.04[Ⓐ]			
GT/TT	27/3	16/9	0.08 [Ⓐ]			
<i>Allele</i>						
G	69 (67.6)	32 (51.5)		2.22	5.4	0.02[Ⓐ]
T	33 (32.4)	34 (48.5)		(1.18–4.2)		
<i>(VDR) genotype</i>						
FF	29 (56.9%)	12 (36.4%)			12.6	0.002[Ⓐ]
Ff	22 (43.1%)	14 (42.4%)				
ff	0 (0.0%)	7 (21.2%)				
FF/Ff	29/22	12/14	0.5 [Ⓐ]			
FF/ff	29/0	12/7	0.002[Ⓐ]			
Ff/ff	22/0	14/7	0.01[Ⓐ]			
<i>Allele</i>						
F	80 (78.4)	38 (57.6)		2.68	7.4	0.006[Ⓐ]
f	22 (21.6)	28 (42.4)		(1.36–5.28)		

Data are number (%).

OR = odds ratio; 95% CI (in parentheses). *P* < 0.05 is considered significant. Bonferroni adjustment of *P* value was considered in multiple comparisons.[Ⓢ] Fisher's exact test.**Table 3**
Demographic, clinical and laboratory characteristics among different GC and VDR genotypes.

Genotypes	GC genotypes			VDR genotypes		
	GG (n = 21)	GT + TT (n = 30)	P value	FF (n = 29)	Ff (n = 22)	P value
Characteristics						
Age, yr	8.3 ± 2.6	8.9 ± 2.8	0.4 [*]	9.1 ± 3.0	8.0 ± 2.2	0.1 [*]
Sex, n (%)						
Male	15 (71.4)	13 (43.3)		16 (55.2)	12 (54.5)	
Female	6 (28.6)	17 (56.7)	0.09 [Ⓢ]	13 (44.8)	10 (45.5)	1.0 [Ⓢ]
Family history, n(%)						
Positive	11 (52.4)	18 (60)		17(58.6)	12 (54.5)	
Negative	10 (47.6)	12 (40)	0.8 [Ⓢ]	12(41.4)	10 (45.5)	0.8 [Ⓢ]
Consanguinity, n (%)						
Positive	11 (52.4)	22 (73.3)		20 (69)	13 (59.1)	
Negative	10 (47.6)	8 (26.7)	0.2 [Ⓢ]	9 (31)	9 (40.9)	0.6 [Ⓢ]
Duration of illness, y	5.5 ± 2.2	6.0 ± 2.9	0.6 [*]	6.2 ± 2.8	5.3 ± 2.5	0.2 [*]
Treatment modality, n (%)						
ICS	18 (85.7)	20 (66.7)		23 (79.3)	15 (68.2)	
No ICS	3 (14.3)	10 (33.3)	0.2 [Ⓢ]	6 (20.7)	7 (31.8)	0.5 [Ⓢ]
Pulmonary functions:						
FEV1 (% predicted)	62.1 ± 5.3	68.8 ± 6	<0.001[*]	64.8 ± 7.2	67.7 ± 5.5	0.1 [*]
FVC	94.1 ± 3.8	94.6 ± 2.6	0.6 [*]	94.7 ± 3.2	94 ± 3.1	0.4 [*]
FEV1/FVC	66 ± 5.1	72.7 ± 5.1	<0.001[*]	68.3 ± 6.4	72 ± 4.8	0.02[*]
Asthma severity, n (%)			<0.001[Ⓢ]			0.4[Ⓢ]
Mild (M)			M/Mod. (0.2 [Ⓢ])			
Moderate (Mod.)	3 (14.3)	17 (56.7)	M/Sev. (0.003[Ⓢ])	9 (31)	11 (50.5)	
Severe (Sev.)	5 (23.8)	10 (33.3)	Mod./Sev. (0.03[Ⓢ])	10 (34.5)	6 (27.3)	
	13 (61.9)	3 (10)		10 (34.5)		
Asthma control, n (%)			<0.001[Ⓢ]			0.4 [Ⓢ]
Controlled (Con.)	3 (14.3)	17 (56.7)	Con./P.Con. (0.7 [Ⓢ])	9 (31)	11 (50)	
Partially controlled (P.Con.)	3 (14.3)	11 (36.7)	Con./Uncon. (0.001[Ⓢ])	10 (34.5)	4 (18.2)	
Uncontrolled (Uncon.)	15(71.4)	2(6.7)	P.Con./Uncon. (0.001[Ⓢ])	10 (34.5)	7 (31.8)	
IgE (IU/mL)	89.6 ± 39.1	57.9 ± 30.4	0.002[*]	78.1 ± 38.1	61.4 ± 35.1	0.1 [*]
Eosinophils count, cells/mm ³	432.8 ± 144.2	386.8 ± 148.4	0.3 [*]	412.2 ± 148.3	397.1 ± 148.3	0.7 [*]
Vitamin D (ng/mL)	22.4 ± 2.5	27.2 ± 2.4	<0.001[*]	24.5 ± 3.2	26.2 ± 3.5	0.08[*]
+ve consanguinity	23.7 ± 2.7	27.0 ± 2.3	0.001[*]	25.8 ± 2.7	26.2 ± 3.3	0.7[*]
–ve consanguinity	21.0 ± 1.3	27.6 ± 2.7	0.001[*]	21.7 ± 2.4	26.2 ± 4.0	0.01[*]

IgE: Immunoglobulin E; FEV1: Forced expiratory volume in 1 s; FVC: Forced vital capacity. Data are mean ± SD for quantitative variables, or number (%) for qualitative ones. *P* < 0.05 is considered significant.Bonferroni adjustment of *P* values was considered in multiple comparisons.^{*} Independent samples *t*-test.[Ⓢ] Fisher's exact test.

vitamin D level. Our results revealed a significant association between GC polymorphism and asthma ($P = 0.017$). In relation to clinical and laboratory characteristics, the GG genotypes was significantly associated with relevant asthma characteristics including decreased pulmonary functions, increased asthma severity and uncontrolled disease status in addition to elevated IgE level and hypovitaminosis D. It is acceptable that rs2282679, located near the actin subdomain III, may affect binding of vitamin D to VDBP [23]. Referring to the pooled analysis of several GWAS studies, it has been shown that carrying the heterozygous risk allele confers higher risk ($OR = 1.83$) for hypovitaminosis D [6]. Wang et al. reported a nearly 50% increased risk for vitamin D insufficiency in association with the rs2282679 risk allele among Caucasians [7]. Signorello et al. reported that the risk for hypovitaminosis D is equally associated with homozygous and heterozygous individuals for the risk allele of rs2282679 in African Americans. Regarding Caucasians, they fail to detect such association which may be referred to the smaller sample size. The contradictory results, in the two different populations, might be attributed to the low baseline levels of vitamin D in African Americans compared to Caucasians where heavy skin melanization acts as a barrier for African Americans to benefit from sunlight-mediated vitamin D synthesis which empowers the genetic variations as a crucial determinant in vitamin D status despite the small sized study [24].

In fact, the VDR gene has two functional translation initiation codons. In the first codon (ATG), T > C variation abolish the first initiation site where the protein product lacks the first three amino acids [25]. Being not related to other VDR polymorphisms, this genetic variation (rs2228570) acquires a unique role [26]. Several studies demonstrated that the shorter form of the protein (424aa) is more active than the long form (427aa) as a transcription factor [25,27]. Regarding VDR polymorphism, the present results showed that homozygous children for the risk allele “F” are 2.68 times more likely to develop asthma, where the “FF” genotype is significantly associated with decreased pulmonary functions as evidenced by decreased FEV1/FVC compared to “Ff” genotypes and with decreased vitamin D level within –ve consanguineous cases. Zmuda et al. reported that the genotypes distribution of VDR (rs2228570) among Caucasians is 45% Ff, 40% FF and 15% ff [28]. Recently, Maalmi et al. detected a profound association between VDR FokI polymorphism and asthma in Tunisian children [29]. However, in the study of Wjst et al. 951 German participants were investigated where no association of FokI variants with asthma was reported [30]. Importantly, VDR and its ligand 125 (OH) 2D and the retinoid X receptor (RXR) form an heterodimer that binds to gene promoter regions thus exerting a feedback regulation on the levels of vitamin D metabolites. This endocrine control to self-regulate vitamin D concentrations may be interrupted if the VDR, RXR, or any component of vitamin D metabolic pathway is affected [31]. Vitamin D controls the transcription of some cytokine genes that are implicated in airway inflammation in asthma; hence, genetic VDR variation that decreased VDR-dependent signaling pathways would influence the transcription of the target genes and ultimately affect asthma [32–34]. On the contrary to our results, a lack of correlation between FokI and 25(OH) D levels was reported in the study of Maalmi et al. [29] and that of Hibler et al. [35]. However, a significant association between FokI and serum vitamin D levels was detected in the study of Smolders et al. The authors reported that the “F” allele was associated with lower winter and summer serum 25(OH) D levels in MS patients, and with lower 25(OH) D levels in healthy controls [36].

Taken together, our results identified a strong association between genetic variants at the VDBP (GC) and VDR genes and asthma in Egyptian asthmatic children. Remarkably, the GC-rs2282679 appears to be a pivotal variant that control GC locus associations

with vitamin D levels in addition to VDR (rs2228570) which is associated with decreased vitamin D level in –ve consanguineous patients. However, larger population-based study of Egyptian children is still needed in order to validate these findings for better asthma control and treatment.

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