

RESEARCH

Open Access



Vitamin D receptor and vitamin D binding protein gene polymorphisms in patients with asthma: a pilot study

Daina Bastyte^{1,2*}, Laura Tamasauskiene^{1,2}, Ieva Golubickaite³, Rasa Ugenskiene³ and Brigita Sitkauskiene¹

Abstract

Background The effects of vitamin D are exerted by interaction with the vitamin D receptor (VDR) and vitamin D binding protein (VDBP). Polymorphisms in VDR or VDBP genes may affect vitamin D levels, influencing the pathogenesis of asthma and atopy. The aim of this study was to investigate the possible association of VDR and VDBP gene single-nucleotide polymorphisms (SNP), 25-hydroxyvitamin D (25(OH)D), blood eosinophils and total IgE level in subjects with asthma in comparison with healthy individuals.

Methods This case-control study enrolled 63 subjects with asthma (45 allergic and 18 non-allergic) and 32 healthy subjects were involved in the study. Sensitization of subjects to inhaled allergens was determined by a skin prick test, lung function was evaluated by spirometry. Blood eosinophil count was determined by standard methods. Serum 25(OH)D and total IgE levels were evaluated by ELISA. Polymorphisms in the VDR and VDBP genes on the 12q13.11 and 4q13.3 chromosomal region were analyzed using TaqMan SNP Genotyping Assay probes.

Results In asthma patients with vitamin D deficiency (< 20 ng/ml) the allele G of rs11168293 of VDR was more common than in those having insufficiency (20–30 ng/ml) of vitamin D (63% and 31%, $p < 0.05$). Moreover, asthmatic subject with rs11168293 G allele has significant higher blood eosinophil count compared to asthmatic without the rs11168293 G allele ($8.5 \pm 12.3\%$ vs. $5.1 \pm 1.5\%$, $p < 0.05$). Significantly higher IgE level was found in subjects with allergic asthma with the allele A of rs7041 on VDBP gene than in those without this allele (540 ± 110 and 240 ± 80 IU/ml, $p < 0.05$).

Conclusions The association of polymorphisms in VDBP and VDR gene, the rs11168293 G allele and the rs7041 A allele, with 25(OH)D, blood eosinophil and total IgE level in asthma, let us suggest that vitamin D, VDR and VDBP gene polymorphisms are important in pathogenesis of asthma despite its form in relation to atopy.

Keywords Asthma, Allergic asthma, Vitamin D, Vitamin D receptor, Vitamin D binding protein, Single nucleotide polymorphism

*Correspondence:

Daina Bastyte
daina.bastyte@lsmu.lt

¹Department of Immunology and Allergology, Lithuanian University of Health Sciences, Kaunas, Lithuania

²Lab of Immunology, Department of Immunology and Allergology, Lithuanian University of Health Sciences, Kaunas, Lithuania

³Department of Genetics and Molecular Medicine, Lithuanian University of Health Sciences, Kaunas, Lithuania



© The Author(s) 2023, corrected publication 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Background

According to the literature approximately around 300 million people worldwide suffer from asthma [1]. Asthma is a chronic airway disease causing shortness of breath, chest tightness and cough [2]. In the majority of patients symptoms could be precipitated by exposure to allergens [3]. Allergen-induced (atopic) asthma is an inherited predisposition to produce IgE in response to exposure to environmental proteins [4]. Moreover, IgE is a marker of atopy and asthma phenotypes that are not related to specific IgE production are classified as non-allergic asthma [5–7]. Also, some asthma endotypes can have either allergic or non-allergic underpinnings and are typically characterized by some degree of eosinophilic airway inflammation [8].

It is known that vitamin D is subject of a great interest in research because of its potential to modulate the immune system from environmental and genetic aspects. Many studies have been made to understand how vitamin D impacts and regulates the immune system, but still not clear the underlying mechanisms of vitamin D impact and whether vitamin D deficiency contributes to asthma pathogenesis¹⁰. Several studies have shown the relation of vitamin D and atopy or asthma [9–11]. It is known that vitamin D beneficially modulates diverse immunologic pathways in heterogeneous asthma endotypes, regulating the actions of immune cells to dampen excessive immune inflammatory responses [12, 13]. Moreover, vitamin D can affect the pathogenesis of asthma by suppressing the response of Th2 lymphocytes and reducing the production of IL-5, thereby decreasing the eosinophil counts and IgE levels. Vitamin D could be a potential regulator of blood eosinophils and serum IgE level. Several studies have shown that lower levels of vitamin D are associated with an increased blood eosinophil count in asthma [14, 15]. Other studies have noted that low vitamin D status is associated with higher IgE levels in atopic conditions [16–19]. On top of that, some studies assert that IgE levels and eosinophil counts can be higher in subjects with vitamin D deficiency or insufficiency than in those with sufficient levels of vitamin D [20–22], although there are controversial findings in the literature [23, 24].

Systemic vitamin D status is determined based on the serum 25-hydroxyvitamin D (25(OH)D) concentration. This metabolite has a long half-life (2 or 3 weeks) and the production of 25(OH)D in the liver is not tightly regulated. The function of vitamin D is applied via proper transport in circulation by vitamin D binding protein (VDBP also known as GC-globulin) [25]. VDBP binds to vitamin D metabolites and regulates the access of vitamin D to tissues and cells [25, 26]. Biologically active vitamin D metabolite and VDBP complex acts by binding to a vitamin D receptor (VDR) which heterodimerizes with the retinoid X receptor alpha (RXR α) [27, 28]. Vitamin D

binding to VDR causes the heterodimer complex become activated which in turn recognizes vitamin D response elements (VDRE) resulting in gene transcription [28].

Previous studies let us suggest that polymorphisms in VDR and VDBP genes may be associated with changes in vitamin D levels and involved in pathological processes of asthma [29–31]. Some genetic studies have confirmed the relation between VDR gene variation and some diseases including asthma and atopic conditions [32, 33]. We hypothesized that polymorphisms in vitamin D pathway genes such as VDR and VDBP may affect vitamin D levels and influence higher IgE levels and eosinophil counts in asthma.

However, the exact mechanisms modulating the role of vitamin D in the pathogenesis of asthma are still a mystery. The aim of this study was to investigate a single nucleotide polymorphism (SNP) of VDR, VDBP gene in asthma cases and comparison with healthy individuals and to evaluate their relation to vitamin D in asthma.

Methods

Study design

This study was carried out at the Hospital of Lithuanian University of Health Sciences Kauno Klinikos included 63 adults (>18 years) with mild to moderate persistent asthma who were diagnosed and classified according to Global Initiative for Asthma (GINA) recommendations and 32 healthy (non-sensitive) adults as a control group. The study was approved by the ethics committee, and informed consent was obtained from all the participants. Blood samples were collected during the period of September – April, from patients with asthma and healthy subjects who do not take vitamin D supplements, without any other conditions that may negatively influence study results. Sensitization to inhaled allergens was determined by skin prick test. Lung function of asthmatic patients was evaluated by spirometry using a CustovitM pneumotachometric spirometer (Custo Med, Ottobrunn, Germany). Blood samples from the subjects were divided into groups: based on sensitization to allergen (-s), samples were divided into allergic and non-allergic asthma groups.

Sample collection and storage

Peripheral venous blood samples were collected from the subject into K3 EDTA tubes for investigation for the separation of DNA and RNA for genomic and expression studies and for eosinophil count. Samples for total IgE and 25(OH)D assay were drawn into serum tubes. The blood specimen was given a personal identifier number that was used to link and maintain the biological information derived. Serum tubes were centrifuged at 3500 rpm for 10 min, serum was separated and frozen at –70 °C for further analysis.

DNA extraction and SNP genotyping

DNA was isolated using the QIAamp DNA blood mini kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. Eight gene polymorphisms in the VDR (rs7975232, rs1544410, rs731236, rs3847987, rs2228570, rs11168293), and VDBP (rs4588, rs7041) genes in chromosome regions 12q13.11 and 4q13.3 were analyzed using TaqMan SNP Genotyping Assays probes (Thermo Fisher Scientific, CA, USA), respectively, according to the manufacturer's protocol.

Evaluation of vitamin D, blood eosinophils and total IgE

Measurements of serum 25(OH)D were performed by the enzyme-linked immunosorbent assay ELISA using DAsource 25OH vitamin D Total ELISA kit (Louvain-la Neuve, Belgium). The analysis kit detection limit was defined as the apparent concentration two standard deviations below the average OD at zero binding was 1,5 ng/mL. Analysis of data was performed according to vitamin D content: deficiency <20 ng/ml (<50 nmol/L), insufficiency 20–30 ng / ml (50–75 nmol/L), normal amount 30–50 ng/ml (75–125 nmol/L) [34–37].

Blood eosinophils evaluation was performed with an automated hematology analyzer (Sysmex, Kobe, Japan).

Measurements of total IgE level in serum were performed by ELISA using commercial test kit (ELISA, Bio-Clin-Inc., St. Louis, USA).

Sample size and statistical analysis

Sample size was estimated according to the data of asthma incidences in the Lithuanian population [38], using standardized sample size calculation formula (minimal number of required study subjects is 30).

All statistical analyses were performed on IBM SPSS Statistics, version 29 (IBM Corp., Armonk, NY, USA) and Microsoft Excel (Microsoft, Redmond, WA, USA).

Methods of statistical analysis were selected after performance of Kolmogorov–Smirnov test. The significance of the differences between the group of patients and the asthma control group was assessed using the Student's t-test (means, Gaussian populations), Mann–Whitney U test (medians, non-Gaussian populations) or Kruskal–Wallis test (medians, non-Gaussian populations), and Fisher's exact test (proportions) were used. P values less than 0.05 were considered as significant in all cases. The odds ratios (OR) were calculated from allelic frequency with 95% confidence interval (95% CI) for the polymorphism of the VDR gene. Power analysis of the study was 60%. Evaluating the association between VDR, VDBP gene SNPs, eosinophil and total IgE level in subject with asthma study power was 80%.

Results

Demography state of studied subject

The demographic characteristics of the study groups are shown in Table 1. Studied groups did not differ significantly according to the study subject age. Subjects with allergic asthma had significantly higher total IgE levels than subjects with non-allergic asthma. There were no significant differences of blood eosinophils and lung function parameters between subjects with asthma despite their allergic status. In this study samples were divided into groups based allergen sensitization status (allergic, non-allergic asthma), vitamin D content (deficiency <20 ng/ml, insufficiency 20–30 ng/ml, normal amount 30–50 ng/ml) and assessed the type of VDR gene polymorphisms and VDBP gene polymorphisms in asthma subjects and control group.

Table 1 Demographic characteristics of the study groups

	Subjects with asthma (n = 63)		
	Allergic asthma (n = 45)	Non-allergic asthma (n = 18)	Control group (n = 32)
Male/female, N	16/29	1/17	13/19
Age, years	41 ± 2	51 ± 2	37 ± 2
Total IgE (kU/L)	420 ± 70*	70 ± 20	N/A
Blood eosinophils (% of leukocytes)	5.9 ± 0.6	4.2 ± 0.7	N/A
Lung function:			
FEV1 (l)	3.2 ± 0.1	2.4 ± 0.2	N/A
FEV1 (% from predicted value)	96.2 ± 2.5	90.1 ± 6.7	N/A
FVC (l)	4.1 ± 0.2	3.2 ± 0.2	N/A
FVC (% from predicted value)	105.4 ± 2.5	104.0 ± 6.9	N/A
FEV1/FVC (ratio)	0.9 ± 0.3	0.8 ± 0.52	N/A

Values are presented as mean ± SEM unless otherwise indicated

*p < 0.05 compared between allergic and non-allergic asthma groups

FEV1 – forced expiratory volume in one second

FVC – forced vital capacity

Table 2 VDR and VDBP polymorphisms (%) in studied groups

VDR gene variant	Genotype	Allergic asthma (n = 45)	Non-allergic asthma (n = 18)	Control group (n = 32)
rs7975232	AA	31	6	31
	AC	42	50	5
	CC	27	44	28
rs1544410	CC	42	65	37
	TC	49	35	50
	TT	9	0	13
rs731236	AA	44	67	36
	AG	49	33	50
	GG	7	0	14
rs3847987	CC	78	78	8
	CA	13	22	16
	AA	9	0	3
rs2228570	GG	27	18	28
	AG	56	53	53
	AA	18	29	19
rs11168293	GG	49	22	38
	GT	31	50	44
	TT	20	28	19
VDBP gene variant	Genotype, major allele	Allergic asthma (n = 45)	Non-allergic asthma (n = 18)	Control group (n = 32)
rs4588	GG	52	53	43
	GT	36	24*	53
	TT	12	24*	3
rs7041	AA	18	25	16
	AC	48	31	52
	CC	35	49	32

*p < 0.05 compared to control group

Table 3 Proportion of subject with asthma according to different serum 25(OH)D level

Serum 25(OH)D level	Allergic asthma (n = 45)	Non-allergic asthma (n = 18)	Control group (n = 32)
Deficiency (< 20 ng/ml)	87 (n = 39)*	83 (n = 15)*	44 (n = 14)
Insufficiency (20–30 ng/ml)	11 (n = 5)*	17 (n = 3)*	50 (n = 16)
Normal (30–50 ng/ml)	2 (n = 1)	0 (n = 0)	6 (n = 2)

*p < 0.01 compared to control group

VDR and VDBP SNP genotyping in asthma group and healthy subjects

In the study were analyzed six SNPs in the VDR (rs7975232, rs1544410, rs731236, rs3847987, rs2228570, rs11168293), and two SNPs in VDBP (rs4588, rs7041) genes in chromosome regions 12q13.11 and 4q13.3 (Table 2). The study revealed that VDBP gene rs4588 GT genotype was less common and TT more common in subjects with non-allergic asthma than in the control group (24 vs. 53 and 24 vs. 3%; respectively, $p < 0.05$). There was no statistically significant difference in VDR gene polymorphisms between study groups. The genotype and allele frequencies of the analyzed VDR and VDBP SNPs in subjects with allergic asthma, non-allergic asthma and control group are presented in Table 2.

Analysis of serum 25(OH)D level

Serum 25(OH)D level was statistically significantly lower in allergic and non-allergic asthma patients than in healthy subjects (14.0 ± 0.8 and 15.4 ± 1.0 vs. 22.6 ± 1.2 ng/ml; respectively, $p < 0.01$). There were no statistically significant differences of 25(OH)D levels between asthma groups despite allergic sensitization. According to 25(OH)D level, its deficiency was found more frequently in patients with allergic and non-allergic asthma compared to the control group (Table 3). Also no significant correlation was found between 25(OH)D level and blood eosinophil count, IgE levels or lung function parameters in any of the studied groups.

The association between VDR and VDBP gene polymorphisms and serum 25(OH)D level

The genotype and allele frequencies of the analyzed VDR and VDBP genes single SNPs in patients with asthma and

control group between different serum 25(OH)D level groups are presented in Table 4. Rs11168293 G allele was more common in subjects with asthma who were found to be deficient in 25(OH)D than in those who were found to have insufficiency of this vitamin. No significant statistical differences were observed between different 25(OH)D level groups and VDR or VDBP gene variants in healthy subjects.

The association between VDR and VDBP gene polymorphisms and blood eosinophils in asthma

Study subjects with asthma and rs11168293 G allele had significantly higher blood eosinophil count (% of leukocytes) compared to asthmatic subjects without the

rs11168293 G allele (Table 5). However, there was no statistically significant difference in genotype and eosinophil count between allergic and non-allergic asthma groups.

The association between VDR and VDBP gene polymorphisms and total IgE level in asthma

Study results revealed that significantly higher IgE levels were found in subjects with allergic asthma with the allele A of rs7041 on VDBP gene than in those without this allele (Table 6). There was no statistically significant difference between VDR SNPs and studied groups.

Table 4 The association between serum 25(OH)D level, genotype and allele frequencies (in %) of VDR and VDBP gene variants in asthmatic subject

VDR gene variant	Genotype, major allele	25(OH)D level asthma group		25(OH)D level control group	
		<20 ng/ml n=5	20–30 ng/ml n=8	<20 ng/ml n=14	20–30 ng/ml n=16
rs7975232	AA	26	13	29	31
	AC	44	38	50	38
	CC	30	50	21	31
	A allele	48	31	54	50
rs1544410	CC	49	50	31	40
	TC	43	50	46	53
	TT	8	0	23	7
	C	71	75	54	67
rs731236	AA	52	50	31	39
	AG	42	50	46	54
	GG	6	0	23	8
	A allele	71	75	54	65
rs3847987	CC	76	88	86	81
	CA	17	13	14	13
	AA	7	0	0	6
	C allele	84	94	93	88
rs2228570	GG	36	38	36	38
	AG	50	50	50	50
	AA	14	13	14	13
	G allele	61	63	61	63
rs11168293	GG	44	13	43	38
	GT	37	38	36	44
	TT	19	50	21	19
	G allele	63*	31	61	59
VDBP gene variant	GENOTYPE, MAJOR ALLELE	25(OH)D LEVEL IN ASTHMA GROUP		25(OH)D LEVEL IN CONTROL GROUP	
		<20 ng/ml n=54	20–30 ng/ml n=8	<20 ng/ml n=14	20–30 ng/ml n=16
rs4588	GG	52	50	42	37
	GT	34	25	58	56
	TT	14	25	0	6
	G allele	69	63	71	66
rs7041	AA	19	29	23	13
	AC	43	29	39	69
	CC	37	43	39	19
	C allele	41	43	42	47

*p<0.05 compared vitamin D deficiency group with vitamin D insufficiency group in patients with asthma

Table 5 The association between alleles of VDR, VDBP gene variants and blood eosinophils count (% of leukocytes) in subjects with asthma

VDR gene variant	Allele	blood eosinophil count (% of leukocytes)	p-Value
rs7975232	A	7.8 ± 1.2	0.22
	C	6.9 ± 1.6	
rs1544410	C	7.3 ± 1.3	0.27
	T	7.7 ± 1.2	
rs731236	A	7.6 ± 1.3	0.80
	G	7.7 ± 1.2	
rs3847987	C	7.7 ± 1.2	0.60
	A	8.5 ± 3.	
rs2228570	G	7.6 ± 1.4	0.81
	A	8.5 ± 1.7	
rs11168293	G	8.5 ± 1.3*	< 0.05
	T	5.1 ± 1.5	
VDBP gene variant	Allele	blood eosinophil count (% of leukocytes)	p-Value
rs4588	G	7.7 ± 2.8	0.58
	T	7.1 ± 2	
rs7041	A	7.2 ± 1.5	0.45
	C	7.4 ± 1.5	

Values are presented as mean ± SEM

* p < 0.05 compared genes different alleles

Table 6 The association between alleles of VDR and VDBP gene variants and total IgE level in subject with asthma

VDR gene variant	Allele	Total IgE level, kU/l	p-Value
rs7975232	A	430 ± 80	0.71
	C	420 ± 90	
rs1544410	C	420 ± 70	0.27
	T	470 ± 100	
rs731236	A	430 ± 90	0.29
	G	500 ± 110	
rs3847987	C	450 ± 70	0.08
	A	110 ± 50	
rs2228570	G	370 ± 70	0.62
	A	370 ± 70	
rs11168293	G	420 ± 80	0.20
	T	320 ± 80	
VDBP gene variant	Allele	Total IgE level, kU/l	p-Value
rs4588	G	400 ± 80	0.19
	T	490 ± 120	
rs7041	A	540 ± 110*	< 0.05*
	C	240 ± 80	

Values are presented as mean ± SEM

* p < 0.05 compared genes different alleles

Discussion

It was demonstrated that serum 25(OH)D level, blood eosinophil or total IgE level in asthma could be related to the single nucleotide polymorphisms in VDR or VDBP gene. We found statistically significant differences in the distribution of rs11168293 genotype variants in the VDR gene between asthma patients with vitamin D deficient (< 20 ng/ml) and asthmatic subjects with vitamin D insufficiency (20–30 ng/ml). Moreover, there was a significantly higher blood eosinophils count in asthmatic with rs11168293 G allele compared to asthma patients without the rs11168293 G allele. We also determined that rs7041

genotype of VDBP gene was related to total IgE level in asthma. There is a possibility that vitamin D and VDR may regulate immune markers that promote asthma.

In this study samples were divided into groups based allergen sensitization status (allergic, non-allergic asthma), vitamin D content (deficiency < 20 ng/ml, insufficiency 20–30 ng/ml, normal amount 30–50 ng/ml) and assessed the type of VDR gene polymorphisms (rs7975232, rs1544410, rs731236, rs3847987, rs2228570, rs11168293) and VDBP gene polymorphisms (rs7041, rs4588) in asthma subjects and control group. We observed that vitamin D deficiency was more frequent

in asthmatics than in healthy subjects. This shows that vitamin D has anti-inflammatory effects and plays an important role in the pathogenesis of asthma. Moreover, several studies have found that low serum vitamin D levels are associated with increased exacerbations, increased airway inflammation, decreased lung function, and poor prognosis in asthmatic patients [39]. Experimental studies of animal models showed immunomodulation effects of vitamin D and VDR involving the Th2-driven inflammation in the lung [40]. In addition, are evidences that decreased vitamin D level induces secretion of IL-5, IL-6, and IL-8 which are important in pathogenesis of inflammation [41]. Also, vitamin D could downregulate the expression of MHC class II molecules and inhibit DC differentiation and maturation by preserving the immature phenotype of dendritic cells [42]. Most observational researchers have shown that vitamin D can be effective as an adjunctive treatment for asthma [43]. On the other hand, the findings of studies are controversial and do not unequivocally support a beneficial role of vitamin D in asthma or atopy [44, 45].

Studies revealed that some polymorphisms variant in VDR and VDBP gene can affect function of the receptor and contribute to circulating vitamin D concentration [46–48]. Hypothetically, low vitamin D levels in asthma and atopy may be associated with SNPs of the VDR and VDBP genes [45, 48–50]. For example, the study of Taiwanese and Mongolian populations demonstrated that Vitamin D plasma concentration lower than 40 ng/ml and VDR SNPs rs2228570 genotype, both contribute to increased susceptibility to bronchial asthma [9]. On top of that, studies revealed that VDR SNPs such as rs2228570 genotype have associations with lower levels of vitamin D [51]. In addition, meta-analysis study revealed that VDR rs7975232, rs1544410 and rs731236 gene polymorphisms may confer susceptibility to allergic diseases in certain populations [52]. Interesting, that asthma meta-analysis study showed positive findings for rs1544410 polymorphism in Caucasians, and for rs731236 polymorphism in Asians [52]. Although, several studies with asthma and atopy showed that some genotypes of VDR are associated with asthma, but not with vitamin D levels [53–55]. Our findings showed that G allele of variant rs11168293 on VDR gene was more common in subjects with asthma who were found to be deficient in vitamin D than in those with vitamin D insufficiency. We did not find statistically significant results between other genotypes of VDR and 25(OH)D levels. On the other hand, Galvao et al. study showed an association of several other polymorphisms in the VDR gene between vitamin D level in asthma and atopy, however in the study was not assessed the polymorphisms of rs11168293 on the VDR gene [33]. Unfortunately, there is a paucity of evidence regarding the relation of rs11168293 with vitamin D

level or asthma. However, there is a possibility that different VDR gene variants may result in alteration of the biological effects produced by the binding of the receptor with vitamin D in the promoter regions of genes that respond to vitamin [56]. It could affect regulatory actions produced by immune systems cells and cause changes in immune markers of asthma. On the other hand, controversial results shows that potential associations of the VDR SNPs with asthma and atopy are unclear.

Study results revealed that asthmatic subjects with rs11168293 genotype G allele had significantly higher blood eosinophil count compared to asthmatic subjects without this allele. We did not find significant results between blood eosinophil count and 25(OH)D level, on the other hand, Filho et al. study showed that eosinophilic inflammation may be associated with lower vitamin D levels [21]. It is important that vitamin D via VDR prolongs eosinophil survival and increases the expression of membrane receptors that inhibit their apoptosis [57]. We hypothesize that such effects of vitamin D on eosinophils may be related to G allele of variant rs11168293 on the VDR gene and may lead to a lower need for new eosinophils. Unfortunately, the variant rs11168293 is poorly explored in vitamin D and eosinophilic inflammation contexts. The rs11168293 genotype of VDR should be further studied on a larger cohort to derive conclusive results.

In our study, we noted statistically significant differences in VDBP gene rs4588 GT and TT genotype in non-allergic asthma compared to the healthy controls. VDBP gene rs4588 GT genotype was less common and TT more common in subjects with non-allergic asthma than in the healthy subjects. On top of that, study of Kurdish population showed increased level of serum VDBP in asthmatic patients compared to the control group and revealed that variations in VDR and VDBP gene may impact on progression of asthma [50]. In addition, Shafi et al. study showed that CC genotype of rs4588 was linked to higher Vitamin D levels whereas the AA genotype was linked to lower levels of vitamin D in subjects with atopic dermatitis [48]. Persic et al. in the study with coronary artery disease patients revealed that VDBP gene rs4588 genotype GG was correlated with higher levels of vitamin D in the serum [58]. However, we found no significant difference between VDBP SNP's and vitamin D levels. Also, we did not find a significant difference in VDBP gene polymorphi [50]. In addition, Iordanidou et al. also did not find an association between serum VDBP rs4588 genotypes and vitamin D level in asthma [59].

It was found that rs7041 genotype variant of VDBP gene was associated with IgE levels in asthma. In addition, Fawzy et al. study revealed that GG genotype and G allele of the rs7041 are potential risk factors for the development of asthma, while rs4588 AA genotype

and A allele may play protective role [60]. Significantly higher IgE levels were found in subjects with allergic asthma with the rs7041 A allele than in those without it. On top of that, German Asthma Family Study found association between VDBP rs222040, rs7041 and total serum IgE [61]. VDBP is not only a carrier protein for vitamin D metabolites, but also involved in inflammation modulation [62, 63]. Several studies showed that VDBP is restricting the bioavailability of vitamin D to immune cells like monocytes, dendritic cells and therefore suppresses vitamin D receptor signaling [64, 65], it is important that VDBP also is associated with membrane-bound immunoglobulin on the surface of B-lymphocytes [66]. The rs7041 A allele of VDBP gene might be associated with allergen-induced inflammation.

We did not find a significant difference between VDR SNP and IgE level. Differently than eosinophils [57] IgE does not have a VDR but it is produced by B lymphocytes, vitamin D via VDR exerting an immunomodulatory effect to lymphocytes and directly or indirectly regulates the production of IgE in B lymphocytes [13, 67]. Hypothetically, that would explain why there is no relation between VDR polymorphisms and IgE.

Discrepancies between studies on VDR, VDBP gene SNP in atopy or asthma may be obtained due to different study populations with geographical and ethnical differences among the subjects involved in these studies. Moreover, the interaction between genes and environmental factors such as sun exposure, diet, or interactions of VDR gene with other genes involved in vitamin D metabolism in asthma and atopy may play an additional role, especially in complex diseases such as asthma. On top of that, inadequate limited sample sizes may also result in discrepancies in findings between the studies. This might be one of the reasons that our results are not in agreement with some other previous studies results.

To our knowledge, it is the first VDR and VDBP polymorphism study of asthma in the Lithuanian population, also there is limited available research data in The European population. Further studies are warranted to investigate the VDR and VDBP gene single nucleotide polymorphisms' role in the immune system and to evaluate their relation with vitamin D level of asthma in different populations.

Conclusions

Increased incidence of serum vitamin D deficiency in subjects with asthma, and association of the rs11168293 G allele on VDR gene with lower 25(OH)D level, higher blood eosinophil count (% of leukocytes) and the rs7041 A allele on VDBP gene with higher IgE level in atopic asthma, let us suggest that vitamin D and a number of its binding protein and receptor gene polymorphisms are important in pathogenesis of asthma.

Abbreviations

ELISA	Enzyme-linked immunosorbent assay
FEV1	Forced expiratory volume in one second
FVC	Forced vital capacity
GINA	Global Initiative for Asthma
IgE	Total immunoglobulin E
IL	Interleukin
SEM	Standard error of mean
SNP	Single nucleotide polymorphisms
Th2	Type 2 helper T cells
VDBP	Vitamin D binding protein
VDR	Vitamin D receptor

Acknowledgements

We are grateful to Dr. Edita Gasiuniene for her help with involving patients to the study and to Rytis Boreika for his help in writing the manuscript.

Author contributions

DB performed and analyzed laboratory tests, performed statistical analysis and was a major contributor in writing the manuscript. LT involved patients into the study, performed clinical investigation. IG and RU performed and evaluated genetic laboratory tests and contributed to explaining the methods of laboratory tests. BS supervised the study and was responsible for manuscript revision. All authors read and approved the final manuscript.

Funding

This study was supported in part by Research Foundation of Lithuanian University of Health Sciences and Research Council of Lithuania (project reg. no. P-LLT—20—4).

Data Availability

All data generated or analyzed during this study are included in this published article.

The data presented in this study are available on request from the corresponding author.

Declarations

Ethics approval and consent to participate

The investigation was conducted in accordance with the Declaration of Helsinki, all methods were performed in accordance with the relevant guidelines and regulations. The study was approved by Regional Bioethics Committee at the Lithuanian University of Health Sciences (No. BE-2-74). Subjects gave their written informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 13 October 2022 / Accepted: 23 June 2023

Published online: 05 July 2023

References

1. Dharmage SC, Perret JL, Custovic A. Epidemiology of asthma in children and adults. *Front Pediatr* 2019 Jun 18;7:246.
2. Posner J, Bradley S, Peterson JAR. Asthma outcomes: asthma symptoms. *J Allergy Clin Immunol*. 2012;23(1):1–7.
3. Knudsen TB, Thomsen SF, Nolte H, Backer V. A Population-based clinical study of allergic and non-allergic asthma. *J Asthma*. 2009 Jan;46(1):91–4.
4. Thomsen SF. Epidemiology and natural history of atopic diseases. *Eur Clin Respir J*. 2015 Mar;24. <https://doi.org/10.3402/ecrj.v2.24642>.
5. Baos S, Calzada D, Cremades-Jimeno L, Sastre J, Picado C, Quiralte J, et al. Nonallergic asthma and its severity: biomarkers for its discrimination in peripheral samples. *Front Immunol*. 2018;9:1416.
6. Lababidi HMS, AlSowayigh OM, BinHowemel SF, AlReshaid KM, Alo-taiq SA, Bahnassay AA. Refractory asthma phenotyping based on

- immunoglobulin E levels and eosinophilic counts: a real life study. *Respir Med*. 2019 Oct;158:55–8.
7. Oettgen HC, Geha RS. IgE regulation and roles in asthma pathogenesis. *J Allergy Clin Immunol*. 2001 Mar 1;107(3):429–41.
 8. Nelson RK, Bush A, Stokes J, Nair P, Akuthota P. Eosinophilic asthma. *J Allergy Clin Immunol Pract*. 2020 Feb;8(1):465–73.
 9. Munkhbayarlakh S, Kao HF, Hou YI, Tuvshintur N, Bayar-Ulzii B, Narantsetseg L et al. Vitamin D plasma concentration and vitamin D receptor genetic variants confer risk of asthma: A comparison study of Taiwanese and Mongolian populations. *World Allergy Organ J [Internet]*. 2019 Nov [cited 2021 Sep 14];12(11). Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6838943/>.
 10. Bener A, Ehlayel MS, Tulic MK, Hamid Q. Vitamin D Deficiency as a strong predictor of Asthma in Children. *Int Arch Allergy Immunol*. 2012;157(2):168–75.
 11. Esfandiari N, Alaei F, Fallah S, Babaie D, Sedghi N. Vitamin D deficiency and its impact on asthma severity in asthmatic children. *Ital J Pediatr*. 2016 Dec;17(1):108.
 12. Di Rosa M, Malaguarnera M, Nicoletti F, Malaguarnera L. Vitamin D3: a helpful immuno-modulator. *Immunology*. 2011 Oct;134(2):123–39.
 13. James J, Weaver V, Cantorna MT. Control of Circulating IgE by the Vitamin D Receptor In Vivo Involves B Cell Intrinsic and Extrinsic Mechanisms. *J Immunol Baltim Md 1950*. 2017 Feb 1;198(3):1164–71.
 14. Souto Filho JTD, de Andrade AS, Ribeiro FM, Alves P, de AS, Simonini VRF. Impact of vitamin D deficiency on increased blood eosinophil counts. *Hematol Oncol Stem Cell Ther*. 2018 Mar 1;11(1):25–9.
 15. Carriero V, Bullone M, Bertolini F, Ricciardolo FLM. Vitamin D negatively correlates with blood eosinophilia in stable adult asthmatics. *Eur Respir J [Internet]*. 2019 Sep 28 [cited 2022 Mar 15];54(suppl 63). Available from: https://erj.ersjournals.com/content/54/suppl_63/PA4305.
 16. Manousaki D, Paternoster L, Standl M, Moffatt MF, Farrall M, Bouzigon E, et al. Vitamin D levels and susceptibility to asthma, elevated immunoglobulin E levels, and atopic dermatitis: a mendelian randomization study. *PLOS Med*. 2017 May;9(5):e1002294.
 17. Sharief S, Jariwala S, Kumar J, Muntner P, Melamed ML. Vitamin D levels and food and environmental allergies in the United States: results from the National Health and Nutrition Examination Survey 2005–2006. *J Allergy Clin Immunol*. 2011 May;127(5):1195–202.
 18. Guo H, Zheng Y, Cai X, Yang H, Zhang Y, Hao L, et al. Correlation between serum vitamin D status and immunological changes in children affected by gastrointestinal food allergy. *Allergol Immunopathol (Madr)*. 2018 Jan;46(1):39–44.
 19. Farhan K, Alswailmi, Sikandar MZ, Shah SIA. Biological roles of vitamin D and immunoglobulin E: implications in allergic Disorders. *Pak J Med Health Sci*. 2020 Oct;1:14:495–8.
 20. Han YY, Forno E, Boutaoui N, Canino G, Celedón JC. Vitamin D insufficiency, TH2 cytokines, and allergy markers in puerto rican children with asthma. *Ann Allergy Asthma Immunol Off Publ Am Coll Allergy Asthma Immunol*. 2018 Oct;121(4):497–498e1.
 21. Souto Filho JTD, de Andrade AS, Ribeiro FM, Alves P, de AS, Simonini VRF. Impact of vitamin D deficiency on increased blood eosinophil counts. *Hematol Oncol Stem Cell Ther*. 2018 Mar;11(1):25–9.
 22. de Amorim CLCG, de Oliveira JM, Rodrigues A, Furlanetto KC, Pitta F. Vitamin D: association with eosinophil counts and IgE levels in children with asthma. *J Bras Pneumol Publicacao Of Soc Bras Pneumol E Tisiologia*. 2020;47(1):e20200279.
 23. Meral G, Uslu A, Yozgatli AU, Tuna HT, Yilmazbas NP, Akçay F, et al. The relationship between vitamin D, asthma and total IgE in children. *Stud Ethno-Med*. 2017 Jan;2(1):91–7.
 24. Tanısi A, Ve O, Allerjik O, Vitamin H, Karşılaştırılması S, Şafak A et al. Comparison of vitamin D levels in allergic patients with and without Asthma. 2020 Jun 13.
 25. Bouillon R, Schuit F, Antonio L, Rastinejad F. Vitamin D binding protein: a historic overview. *Front Endocrinol* 2020 Jan 10;10:910.
 26. Chun RF, Shieh A, Gottlieb C, Yacoubian V, Wang J, Hewison M et al. Vitamin D Binding Protein and the Biological Activity of Vitamin D. *Front Endocrinol [Internet]*. 2019 [cited 2022 Jun 22];10. Available from: <https://www.frontiersin.org/article/https://doi.org/10.3389/fendo.2019.00718>.
 27. Penna G, Roncarì A, Amuchastegui S, Daniel KC, Berti E, Colonna M, et al. Expression of the inhibitory receptor ILT3 on dendritic cells is dispensable for induction of CD4 + Foxp3 + regulatory T cells by 1,25-dihydroxyvitamin D3. *Blood*. 2005 Nov;15(10):3490–7.
 28. Penna G, Adorini L. 1 Alpha,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. *J Immunol Baltim Md 1950*. 2000 Mar 1;164(5):2405–11.
 29. Navas-Nazario A, Li F, Shabanova V, Weiss P, Cole DE, Carpenter TO, et al. Effect of vitamin D binding protein (DBP) genotype on the development of Asthma in Children. *Ann Allergy Asthma Immunol Off Publ Am Coll Allergy Asthma Immunol*. 2014 Jun;112(6):519–24.
 30. Chen Y, Xu T. Association of vitamin D receptor expression with inflammatory changes and prognosis of asthma. *Exp Ther Med*. 2018 Dec;16(1):5096–102.
 31. Hou C, Zhu X, Chang X. Correlation of vitamin D receptor with bronchial asthma in children. *Exp Ther Med*. 2018 Mar;15(3):2773–6.
 32. Zhang W, Xu Y. Association between vitamin D receptor gene polymorphisms rs2228570 and allergic Rhinitis. *Pharmacogenomics Pers Med* 2020 Aug 17;13:327–35.
 33. Galvão AA, de Araújo Sena F, de Andrade Belitardo EMM, de Santana MBR, Costa GN, de Cruz O. Genetic polymorphisms in vitamin D pathway influence 25(OH)D levels and are associated with atopy and asthma. *Allergy Asthma Clin Immunol*. 2020 Jul;9(1):62.
 34. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Guidelines for preventing and treating vitamin D deficiency and insufficiency revisited. *J Clin Endocrinol Metab*. 2012 Apr;97(4):1153–8.
 35. Pludowski P, Karczmarewicz E, Bayer M, Carter G, Chlebna-Sokół D, Czech-Kowalska J, et al. Practical guidelines for the supplementation of vitamin D and the treatment of deficits in Central Europe - recommended vitamin D intakes in the general population and groups at risk of vitamin D deficiency. *Endokrynol Pol*. 2013;64(4):319–27.
 36. Bauer P, Henni S, Dörr O, Bauer T, Hamm CW, Most A. High prevalence of vitamin D insufficiency in professional handball athletes. *Phys Sportsmed* 2019 Jan 2;47(1):71–7.
 37. Kardelen AD, Kardelen AD. Serum 25(OH) Vitamin D Levels of Adolescent and Young Medical Students. [cited 2021 Dec 19]; Available from: <https://clinmedjournals.org/articles/ijpr/international-journal-of-pediatric-research-ijpr-4-032.php?jid=ijpr>.
 38. Lietuvos sveikatos statistika / Health Statistics of Lithuania. - Higienos institutas [Internet]. [cited 2023 Feb 6]. Available from: <https://www.hi.lt/lt/lietuvos-sveikatos-statistika-health-statistics-of-lithuania.html>.
 39. Hall SC, Agrawal DK. Vitamin D and bronchial asthma: an overview of the last five years. *Clin Ther*. 2017 May;39(5):917–29.
 40. Wittke A, Weaver V, Mahon BD, August A, Cantorna MT. Vitamin D receptor-deficient mice fail to develop experimental allergic Asthma1. *J Immunol*. 2004 Sep;173(1):3432–6.
 41. Poon M, Craig ME, Kaur H, Cusumano J, Sasongko MB, Wong TY, et al. Vitamin D deficiency is not associated with changes in retinal geometric parameters in young people with type 1 diabetes. *J Diabetes Res*. 2013;2013:280691.
 42. Aranow C. Vitamin D and the Immune System. *J Investig Med*. 2011 Aug 1;59(6):881–6.
 43. Ali NS, Nanji K. A review on the role of vitamin D in Asthma. *Cureus* 9(5):e1288.
 44. Denlinger LC, King TS, Cardet JC, Craig T, Holguin F, Jackson DJ, et al. Vitamin D supplementation and the risk of Colds in patients with asthma. *Am J Respir Crit Care Med*. 2016 Mar;193(6):634–41.
 45. Jensen ME, Mailhot G, Alos N, Rousseau E, White JH, Khamessan A et al. Vitamin D intervention in preschoolers with viral-induced asthma (DIVA): a pilot randomised controlled trial. *Trials* 2016 Jul 26;17(1):353.
 46. Tuncel G, Temel SG, Ergoren MC. Strong association between VDR FokI (rs2228570) gene variant and serum vitamin D levels in Turkish Cypriots. *Mol Biol Rep*. 2019 Jun 1;46(3):3349–55.
 47. Valdivielso JM, Fernandez E. Vitamin D receptor polymorphisms and diseases. *Clin Chim Acta Int J Clin Chem*. 2006 Sep;371(1–2):1–12.
 48. Shafi T, Farooq I, Bhat IA, Rasool R, Sameem F, Rashid I et al. Delineating the relationship of Vitamin D binding protein (VDBP) genetic variants in exon 11 with serum Vitamin D levels in Atopic Dermatitis. *Hum Gene*. 2023 May 1;36:201178.
 49. Zhang W, Xu Y. Association between vitamin D receptor gene polymorphisms rs2228570 and allergic Rhinitis. *Pharmacogenomics Pers Med* 2020 Aug 17;13:327–35.
 50. Nasiri-Kalmarzi R, Abdi M, Hosseini J, Tavana S, Mocarizadeh A, Rahbari R. Association of vitamin D genetic pathway with asthma susceptibility in the kurdis population. *J Clin Lab Anal*. 2020;34(1):e23039.
 51. Tamasauskiene L, Golubickaitė I, Ugenskiene R, Sjakste N, Pamonova N, Wu LSH et al. Vitamin D receptor gene polymorphisms in atopy. *Immun Inflamm*

- Dis [Internet]. [Cited 2021 Sep 14];n/a(n/a). Available from: <https://onlinelibrary.wiley.com/doi/abs/https://doi.org/10.1002/iid3.487>.
52. Zhang L, Zhang S, He C, Wang X. VDR gene polymorphisms and allergic Diseases: evidence from a Meta-analysis. *Immunol Invest*. 2020 Feb;49(1–2):166–77.
 53. Despotovic M, Jevtovic Stojmenov T, Stankovic I, Basic J, Pavlovic D. Vitamin D receptor gene polymorphisms in serbian patients with bronchial asthma: a case-control study. *J Cell Biochem*. 2017;118(11):3986–92.
 54. Papadopoulou A, Kouis P, Middleton N, Kolokotroni O, Karpathios T, Nicolaidou P, et al. Association of vitamin D receptor gene polymorphisms and vitamin D levels with asthma and atopy in cypriot adolescents: a case-control study. *Multidiscip Respir Med*. 2015;10(1):26.
 55. Kilic M, Ecin S, Taskin E, Sen A, Kara M. The vitamin D receptor gene polymorphisms in Asthmatic Children: a case-control study. *Pediatr Allergy Immunol Pulmonol*. 2019 Jun;32(1):63–9.
 56. Nurminen V, Seuter S, Carlberg C. Primary Vitamin D. Target Genes of Human Monocytes. *Front Physiol* [Internet]. 2019 [cited 2022 Jan 23];10. Available from: <https://www.frontiersin.org/article/https://doi.org/10.3389/fphys.2019.00194>.
 57. Hiraguchi Y, Tanida H, Sugimoto M, Hosoki K, Nagao M, Tokuda R, et al. 1,25-Dihydroxyvitamin D3 upregulates functional C-X-C chemokine receptor type 4 expression in human eosinophils. *Int Arch Allergy Immunol*. 2012;158(Suppl 1):51–7.
 58. Peršić V, Raljević D, Markova-Car E, Cindrić L, Miškulin R, Žuvić M, et al. Vitamin D-binding protein (rs4588) T/T genotype is associated with anteroseptal myocardial infarction in coronary artery disease patients. *Ann Transl Med*. 2019 Aug;7(16):374.
 59. Iordanidou M, Paraskakis E, Giasari G, Manolopoulos V, Chatzimichael A. VDR and VDBP polymorphisms are associated with 25(OH)D3 levels in asthmatic children. *Eur Respir J* [Internet]. 2014 Sep 1 [cited 2022 Jan 23];44(Suppl 58). Available from: https://erj.ersjournals.com/content/44/Suppl_58/P4211.
 60. Fawzy MS, Elgazzaz MG, Ibrahim A, Hussein MH, Khashana MS, Toraih EA. Association of group-specific component exon 11 polymorphisms with bronchial asthma in children and adolescents. *Scand J Immunol*. 2019 Mar;89(3):e12740.
 61. Wjst M, Altmüller J, Faus-Kessler T, Braig C, Bahnweg M, André E. Asthma families show transmission disequilibrium of gene variants in the vitamin D metabolism and signalling pathway. *Respir Res* 2006 Apr 6;7(1):60.
 62. Bratke K, Wendt A, Garbe K, Kuepper M, Julius P, Lommatzsch M, et al. Vitamin D binding protein and vitamin D in human allergen-induced endobronchial inflammation. *Clin Exp Immunol*. 2014 Jul;177(1):366–72.
 63. Bikle DD, Schwartz J, Vitamin D, Binding Protein. Total and free vitamin D levels in different physiological and pathophysiological conditions. *Front Endocrinol*. 2019 May;28:10:317.
 64. Chun RF, Lauridsen AL, Suon L, Zella LA, Pike JW, Modlin RL, et al. Vitamin D-binding protein directs monocyte responses to 25-hydroxy- and 1,25-dihydroxyvitamin D. *J Clin Endocrinol Metab*. 2010 Jul;95(7):3368–76.
 65. Jeffery LE, Wood AM, Qureshi OS, Hou TZ, Gardner D, Briggs Z et al. Availability of 25-hydroxyvitamin D(3) to APCs controls the balance between regulatory and inflammatory T cell responses. *J Immunol Baltim Md 1950*. 2012 Dec 1;189(11):5155–64.
 66. Petrini M, Allegrini A, Ambrogi F, Valentini P, Sabbatini A, Arnaud P, et al. Binding of GC (VDBP) to membranes of human B lymphocytes following stripping of extant protein. *J Endocrinol Invest*. 1995 Sep;18(8):630–7.
 67. Hartmann B, Heine G, Babina M, Steinmeyer A, Zügel U, Radbruch A et al. Targeting the vitamin D receptor inhibits the B cell-dependent allergic immune response. *Allergy* 2011 Apr;66(4):540–8.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.