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ASTHMA

Association Between Vitamin D Metabolism Gene Polymorphisms and Risk of Tunisian Adults' Asthma

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Abstract

Introduction Several studies have shown a strong correlation between the serum vitamin D level and asthma severity and deficits in lung function.

Objective Study the relationship between vitamin D and the severity of asthma by targeting five SNPs of vitamin D metabolism gene pathway in a Tunisian adult asthmatics population.

Methods Our case–control study includes 154 adult asthmatic patients and 154 healthy Tunisian subjects. We genotyped many variants in three human genes encoding key components of the vitamin D metabolism, CYP2R1, CYP27B1, GC. The GC gene rs4588 and rs7041 polymorphisms were analysed using the PCR-RFLP method, while rs10741657 and rs12794714 for CYP2R1 gene and rs10877012 of CYP27B1 gene were investigated using TaqMan PCR genotyping techniques.

Results We found that the presence of at least one copy of the rs12794714 A, allele was associated with lower risk of developing asthma (OR 0.61). Further, the rs12794714 is a protector factor against asthma severity (OR 0.5). However, the presence of rs10877012 TG genotype is a risk factor related to asthma severity (OR 1.89). When we classified the population according to sex, our results showed that rs10877012 TT genotype was a risk factor for women subjects (OR 6.7). Moreover, the expression of TT genotype was associated with a higher risk of asthma in non-smoker patients (OR 7.13). We found a significant lower VD serum levels in asthmatics than controls but no impact of the polymorphisms on VD levels.

Conclusions We found that rs12794714 and rs10877012 SNPs were associated with asthma risk.

Keywords Pcr-RFLP · TaqMan · CYP2R1 · CYP27B1 · GC · Asthma

Introduction

Asthma is a complex inflammatory disease characterized by recurrent chronic airway obstruction [1]. Vitamin D deficiency has been described to be frequent in inflammatory bowel diseases and associated with altered lung structure and function [1, 2]. It is also associated with many chronic lung diseases, including lung fibrosis, chronic obstructive pulmonary disease (COPD), acute airway infection and asthma severity [3–6].

Vitamin D3 (VitD3) is a prohormone obtained from the dietary sources or metabolized by ultraviolet B (UVB) irradiation of 7-dehydrocholesterol. VitD3 is then converted to its major form in the body, 25(OH)D, by the microsomal cytochrome P450 enzyme (CYP2R1) with vitamin D 25-hydroxylase activity in the liver. Active vitamin D [1,25(OH)2D3] is synthesized locally by the action of Cytochrome P450 Family 27 Subfamily B Member 1 (CYP27B1) in the kidney, although emerging evidence reveals that conversion also occurs locally in tissues, which is likely to be important for immunomodulation [7]. Then, the active form of vitamin D engages the vitamin D receptor (VDR) and members of the retinoic X receptor (RXR) family. This complex translocated to the nucleus, where it binds the vitamin D response element (VDRE) in the promoter of genes to alter their transcription. Rapid inactivation of vitamin D by UVB irradiation and by Cytochrome P450 Family 24 Subfamily A Member 1 (CYP24A1) helps to control its

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activity [8]. Actually, genetic and functional studies point to a key role for vitamin D in asthma and atopy, by studying genes involved in vitamin D pathway.

We chose three genes encoding key components of the vitamin D pathway, which include CYP2R1, CYP27B1 and Group-Specific Component (GC, vitamin D-Binding Protein). These genes are localized on chromosome 11p15.2, 12q14.1 and 4q13.3, respectively. They show an important association with many diseases like polycystic ovary syndrome, COPD, vitamin D-dependent rickets type 1 and asthma [9–12]. Common single nucleotide polymorphisms (SNPs) in these genes (rs4588 and rs7041 of CG, rs12794714 and rs10741557 of CYP2R1 and rs10877012 of CYP27B1) are extensively studied with different pathologies. Their associations with serum vitamin D level and/or asthma severity were also investigated [13, 14].

We aimed to study whether the genetic background plays a role in the relationship between vitamin D and the severity of asthma by targeting five SNPs of vitamin D metabolism gene pathway in a Tunisian adult asthmatics population. We also studied the VD expression levels in correlation with asthma disease in our population.

Materials and Methods

The present study includes 154 patients with bronchial asthma (128 women and 26 men, mean age 45.5 years), recruited from the Department of Pneumology and Respiratory Diseases, Abderrahmane Mami Hospital of Chest Diseases, Ariana, Tunisia. Asthma diagnosis was established according to GINA (The Global Initiative for Asthma) recommendations [15], and patients' selection criteria were developed with a specialized physician (cases with osteoporosis, kidney diseases, pregnant and vitamin D-fortified food were excluded). All patients were subjected to full history taking to identify a family history of asthma, age of onset of the disease, sun exposure and smoking habitude. Clinical examination was done to most subjects including calculation of body mass index (BMI).

The control group consisted of 154 non-asthmatic adults from the National Center of Blood Transfusion. Subjects had no family history of asthma, free from symptoms of other pulmonary diseases, allergy or chronic issues and were age matched with patients.

The characteristics of the study sample are presented in Table 1. All participants had given written informed consent. The local ethics committee of the Medical Faculty of Tunis approved the project.

Table 1 Clinical and demographic features of patients and healthy controls

Variable	Case	Control
Gender, *n* (%)		
Women	128 (83.11)	88 (57.14)
Age, mean ± SD	45.57 ± 13.08	46.36 ± 10.03
BMI, mean ± SD	28.62 ± 6.28	26.72 ± 3.82
Age at onset, mean ± SD	25.67 ± 13.98	–
Severity of asthma[a], *n* (%)		
Mild	19 (12.92)	–
Moderate	67 (45.57)	–
Severe	61 (41.49)	–
Smoking, *n* (%)		
Non-smoker	82 (53.24)	78 (50.64)
Passive	53 (34.41)	35 (22.72)
smoker	19 (12.33)	41 (26.62)
Atopy[a], *n* (%)		
Non-atopy	33 (38.37)	154 (100.0)
Atopy	53 (61.62)	–
Asthma history, *n* (%)		
Yes	50 (32.46)	–
No	104 (67.53)	154 (100.0)
Socio-economic status[a], *n* (%)		
Good	8 (10)	21 (23.86)
Medium	48 (60)	45 (51.13)
Bad	24 (30)	22 (25)
Education[a], *n* (%)		
< 6	21 (30)	26 (30.58)
[6–12]	36 (51.42)	35 (41.17)
> 12	13 (18.57)	24 (28.23)
Sun exposure[a], *n* (%)		
Indoor	31 (40.78)	22 (24.44)
Outdoor	45 (59.20)	68 (75.55)
Associated diseases, *n* (%)		
Yes	94 (61.03)	–
No	60 (38.96)	154 (100.0)
FEV1 (%) predicted, mean ± SD	83 ± 19	–
CVF (%)	88	–
FEV/CVF (%)	79	–

[a]Missing data

SNP Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using the salting out procedure as described [16]. The concentration and purity of extracted DNA, were measured using NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA).

The investigated polymorphisms of GC gene were analysed using the polymerase chain reaction-restriction

fragment length polymorphism (PCR-RFLP) method. Primer sequences used for the amplification of rs4588 and rs7041 polymorphisms was forward primer 5′-AAA TAATGAGCAAATGAAAGAAGAC-3′ and reverse primer 5′-CAATAACAGCAAAGAAATGAGTAGA-3′. The PCR was performed at 95 °C for 15 min, followed by 30 cycles at 94 °C for 45 s, at 51 °C for 45 s, and 72 °C for 45 s. A final extension step was carried out at 72 °C for 7 min. Amplified products (483 pb) were checked by electrophoresis on 2% agarose gel stained with ethidium bromide and compared with the 100-bp DNA size marker (Bioron, Germany). Then, 5 µl of the amplified products were digested, at 37 °C overnight, in a 20 µl reaction containing either 0.25 µl *Hae*III or 0.25 µl *Sty*I restriction enzymes (Fermentas, Germany) for both rs4588 and rs7041 polymorphisms, respectively. RFLP products for rs4588 C>A (Thr436Lys) were 297 and 186 pb for wild-type homozygous genotype, and no restriction (483 pb) for the homozygous mutant genotype, and were 305 and 178 pb for the mutant homozygous and no restriction for the wild-type homozygous genotype for rs7041 T>G (Asp-432Glu) SNP.

CYP2R1 rs10741657 G>A, rs12794714 G>A and CYP27B1 rs10877012 G>T polymorphisms were performed using TaqMan PCR genotyping. All primers and probes used in this study were designed and validated by Applied Biosystems (Foster City, CA). Amplification reactions were performed in a 10 µL final volume in optical 96-well plates. PCR was carried out with 2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C using a 7300 real-time thermocycler PCR system (Applied Biosystems). To validate the TaqMan assays, we randomly selected 20 SNPs samples to be genotyped using standard amplified restriction fragment length polymorphism analysis.

Quantification of VD Levels

Blood withdrawal was performed between October and December 2017. The collected serum was stored at −20 °C. Serum concentrations of 25(OH)D were measured with a radioimmunoassay kit (DiaSorin, Stillwater, MN, USA). This technique is based on a competitive radioimmunoassay using ^{125}I-labelled 25OHD and antibody to 25OHD. A second antibody is used as precipitating agent. The primary antibody recognizes $25OHD_2$ and $25OHD_3$ equally.

Statistical Analysis

Hardy–Weinberg equilibrium was tested in cases and controls separately. Association analysis was performed using standard Chi-squared and Fisher's exact test to detect differences in genotypes and alleles distribution among our groups. We used Epi Info Version 7 (Epistat Statistical Package, Epistat Services, Richardson, TX) and SPSS.V20 software. A *P* value of 0.05 or less was considered statistically significant. The strength of a gene association is indicated by the odds ratio (OR) with a 95% confidence interval (CI). The odds ratio and the 95% confidence intervals (CI) were calculated whenever applicable. ANOVA test and *t* test were performed to investigate the association between CYP2R1, CYP27B1 and GC genotypes with VD measurements.

Results

After genotyping of our five SNPs, only the rs12794714 was associated with asthma risk. We found that the presence of at least one copy of the rs12794714 allele was associated with lower risk of developing asthma [OR 0.61, CI (0.38–0.96), $P = 0.03$] (Table 2). The rs12794714 polymorphism was a protector factor against asthma. We did not find any association between rs4588, rs7041, rs10741657 and rs10877012 polymorphisms with asthma ($P > 0.05$).

The presence of the rs12794714 SNP was associated with a decreased risk of developing asthma in overweight patients' groups. The presence of rs12794714 allele protects overweight subjects from developing asthma [OR 0.30, CI (0.10–0.88), $P = 0.02$] (Supplemental Table 1).

We compared the association between asthma severity and SNPs' genotypes and we found that the rs12794714 was a protector SNP against asthma severity (protector to having severe asthma [OR 0.51, CI (0.27–0.95), $P = 0.03$)]. However, having the TG genotype in rs10877012 of CYP27B1 gene was a risk factor for developing mild or moderate asthma for healthy controls [OR 1.89, CI (1.09–3.29), $P = 0.02$] (Table 3).

We compared asthmatics living in the urban zone and asthmatics lived in the rural zone, our results showed that the rs4588 genotype AC was a risk factor for asthmatics who were living in the urban zone [OR 3.18, CI (1.11–9.48), $P = 0.02$] (Table 4). We can explain that by the existence of pollution, humidity and less sun exposure in the urban zone.

According to Supplemental Table 4 the presence of rs4588 SNP was associated with lower risk of atopic asthma development [OR 0.36, CI (0.13–0.96), $P = 0.04$] and in particular atopy related to mites [OR 0.31, CI (0.09–0.98), $P = 0.04$].

The stratification according to the age of diagnosis indicated that subjects aged between 18 and 46 years old, with at least one copy of rs10877012 T allele had an increased risk of developing asthma [OR 2.00, CI (1.04–3.88), $P = 0.03$] (Supplemental Table 2).

When we classified the population according to sex, our results showed that rs10877012 was a risk factor for women subjects [OR 2.81, CI (1.58–5.05), $P = 0.0003$]

Table 2 Allele frequencies and genotype distribution of rs 4588, rs7041, rs10743657, rs12794714 and rs10877012 polymorphisms among Tunisian asthmatics and control subjects

SNPs	Genotypes	Allele	Population			%	OR (95% CI)	*P*
			Cases	%	Controls			
rs4588	CC		59	38.31	55	35.71	1	
	AC		66	42.85	67	43.50	0.91 (0.55–1.51)	0.73
	AA		29	18.83	32	20.77	0.84 (0.45–1.58)	0.59
	AC+AA		95	61.68	99	64.27	0.89 (0.56–1.42)	0.63
		C	184	59.74	177	57.46	1	
		A	124	40.25	131	42.53	0.91 (0.66–1.25)	0.56
rs7041	TT		111	72.07	118	76.62	1	
	TG		37	24.02	32	20.77	1.22 (0.71–2.11)	0.45
	GG		6	3.89	4	2.59	1.59 (0.42–6.54)	0.47
	TG+GG		43	27.91	36	23.36	1.26 (0.75–2.12)	0.36
		T	259	84.09	268	87.01	1	
		G	49	15.90	40	12.98	1.26 (0.80–1.99)	0.3
rs12794714	GG		69	44.80	51	33.11	1	
	AG		69	44.80	83	53.89	**0.61 (0.37–0.99)**	**0.04**
	AA		16	10.38	20	12.98	0.59 (0.27–1.26)	0.16
	AG+AA		85	55.18	103	66.87	**0.61 (0.38–0.96)**	**0.03**
		G	207	67.20	185	60.06	1	
		A	101	32.79	123	39.93	0.73 (0.52–1.02)	0.06
rs10741657	GG		73	47.40	88	57.14	1	
	AG		60	38.36	52	33.76	1.38 (0.85–2.26)	2.26
	AA		21	13.63	14	9.09	1.80 (0.85–3.87)	0.11
	AA+AG		81	51.99	66	42.85	1.47 (0.94–2.32)	0.08
		G	206	66.88	228	74.02	1	
		A	102	33.11	80	25.97	1.41 (0.99–2.00)	0.052
rs10877012	GG		73	47.40	88	57.14	1	
	TG		60	38.96	52	33.76	1.39 (0.85–2.25)	0.18
	TT		21	13.63	14	9.09	1.80 (0.85–3.80)	0.11
	TG+TT		81	52.59	66	42.85	1.47 (0.94–2.31)	0.08
		G	206	66.88	228	74.02	1	
		T	102	33.11	80	25.97	1.41 (0.99–2.00)	0.052

Bold values indicate statistically significant

Table 3 Association between rs12794714 and rs10877012 and asthma severity

SNPs	Genotypes	Mild + moderate	Severe	Control	OR1 (95% CI)	*P*	OR2 (95% CI)	*P*
rs12794714	GG	32	28	51	1		1	
	AG	39	24	83	0.74 (0.41–1.34)	0.33	0.52 (0.27–1.01)	0.051
	AA	8	5	20	0.64 (0.23–1.60)	0.34	0.45 (0.13–1.31)	0.15
	AG+AA	47	29	103	0.72 (0.41–1.28)	0.26	**0.51 (0.27–0.95)**	**0.03**
rs10877012	GG	33	29	88	1		1	
	TG	37	19	52	**1.89 (1.05–3.40)**	**0.03**	1.10 (0.55–2.17)	0.76
	TT	10	8	14	1.89 (0.74–4.72)	0.15	1.72 (0.62–4.53)	0.26
	TG+TT	47	27	66	**1.89 (1.09–3.29)**	**0.02**	1.24 (0.66–2.30)	0.49

Bold values indicate statistically significant

OR1 mild + moderate vs. control, *OR2* severe vs. control

Table 4 Association between geographic localization and rs4588, among Asthmatic subjects

SNP	Genotypes	Urban		Rural		OR	*P*
		Case (%)	Control (%)	Case (%)	Control (%)		
rs 4588	CC	19 (31.66)	28 (32.94)	12 (60)	5 (41.66)	1	
	AC	26 (43.33)	36 (42.35)	5 (25)	5 (41.66)	**3. 22 (0.98–11. 72)**	**0.04**
	AA	15 (25)	21 (24.70)	3 (15)	2 (16.66)	3.08 (0.76–15.83)	0.11
	AC+AA	41 (68.33)	57 (67.05)	8 (40)	7 (58.33)	**3. 18 (1.11–9. 48)**	**0.02**

Bold values indicate statistically significant

OR case urban (case) vs. case rural (control)

Table 5 Association between subjects' sex, rs12794714 and rs10877012 with asthma risk

SNPs	Genotypes	Women		OR1 (CI)	*P*	Men		OR2 (CI)	*P*
		Case (%)	Control (%)			Case (%)	Control (%)		
rs12794714	GG	54 (41.86)	32 (38.55)	1		15 (60)	19 (26.76)	1	
	AG	63 (48.83)	39 (46.98)	0.95 (0.52–1.73)	0.88	6 (24)	44 (61.97)	**0.17 (0.05–0.51)**	**0.0009**
	AA	12 (9.30)	12 (14.45)	0.59 (0.23–1.50)	0.26	4 (16)	8 (11.26)	0.63 (0.14–2.55)	0.51
	AG+AA	75 (58.13)	51 (39.53)	0.87 (0.49–1.53)	0.63	10 (40)	52 (73.23)	**0.24 (0.09–0.64)**	**0.002**
rs10877012	GG	55 (42.30)	56 (43.07)	1		18 (75)	32 (45.07)	1	
	TG	55 (42.30)	24 (18.46)	**2.32 (1.26–4.31)**	**0.005**	5 (20.83)	28 (39.43)	**0.32 (0.09–0.95)**	**0.03**
	TT	20 (15.38)	3 (3.61)	**6.70 (2.04–29.75)**	**0.001**	1 (4.16)	11 (15.49)	0.16 (0.007–1.09)	0.06
	TG+TT	75 (57.69)	27 (32.53)	**2.81 (1.58–5.05)**	**0.0003**	6 (25)	39 (54.92)	**0.27 (0.09–0.76)**	**0.01**

Bold values indicate statistically significant

OR1 asthmatic women vs. healthy women, *OR2* asthmatic men vs. healthy men

(Table 5). The presence of TT genotype (OR 6.7) confers a higher risk than the TG genotype (OR 2.32). Therefore, the expressions of rs12794714 and rs10877012 SNPs were associated with a decreased risk of asthma development for the men's groups OR < 1.

The subdivision according to smoking habit revealed that the presence of the rs10877012 TT genotype was associated with a higher risk of asthma development for the non-smoker patients [OR 7.13, CI (2.46–23.70), $P = 0.0001$] (Supplemental Table 3). In case of smoker subjects, the heterozygous genotype AG of rs12794714 SNP was a protective factor against asthma risk [OR 0.24, CI (0.06–0.87). P = 0.02]. In addition, the rs10741657 AG or AA genotypes were associated with a decreased risk against air pollution.

We found that the mean serum levels of 25(OH)D in asthmatic patients (19.28 ± 3.82 ng/mL) were significantly lower than controls (28.15 ± 4.67 ng/mL, $P < 0.001$) (Fig. 1).

This significant 25(OH)D level variation between cases and controls was not modified by genetic factors or gender, age, BMI, sex smoking, socio-economic status, education and sun exposure ($P < 0.05$). We did not observe any significant difference in VD levels according to atopy and severity in our population ($P > 0.05$).

We also made an intra and an inter comparisons in VD levels between different groups in our population, according to different SNPs studied. However, the significant distribution of VD serum level between patient and control groups was not influenced by rs10741657, rs12794714, rs10877012 and rs4588 or rs7041SNPs (Figs. 2, 3, 4).

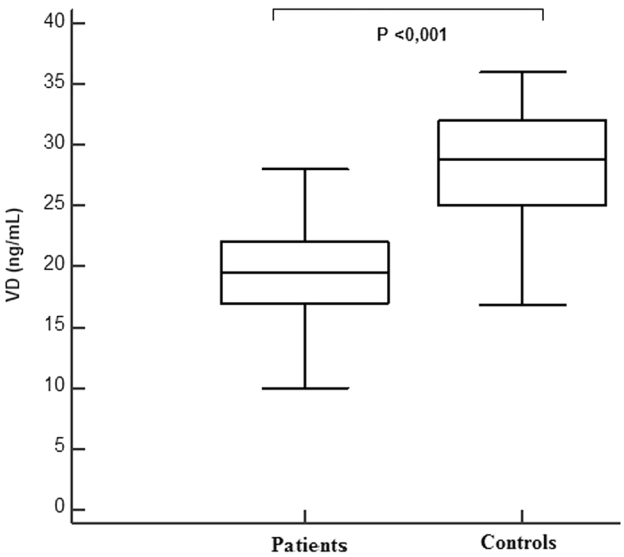

Fig. 1 The distribution of serum vitamin D levels between patients and controls. We found that the mean serum levels of 25(OH)D in asthmatic patients (19.28 ± 3.82 ng/mL) were significantly lower than controls (28.15 ± 4.67 ng/mL, $P < 0.001$)

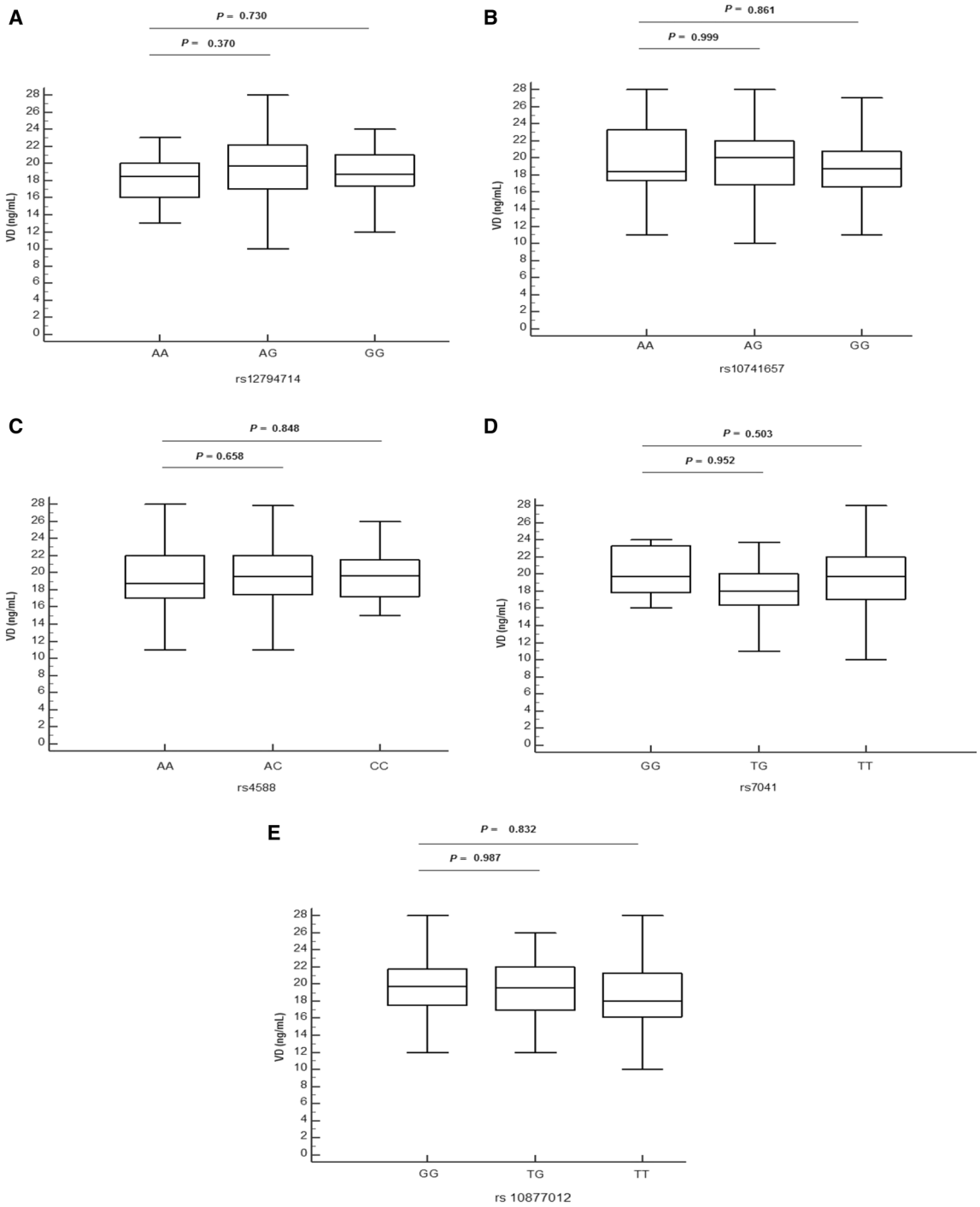

Fig. 2 The distribution of serum vitamin D levels among patients according to VD gene metabolism pathway. The distribution of serum vitamin D levels among patients according to: **a** rs12794714 genotypes, **b** rs10741657 genotypes, **c** rs4588 genotypes, **d** rs7041 genotypes and **e** rs10877012 genotypes

Discussion

The aim of this study was to investigate the association between five polymorphisms in the GC gene (rs7041 and rs4588), CYP2R1 gene (rs10741657 and rs12794714) and CYP27B1 gene (rs10877012), and risk of asthma among Tunisian adults. We stratified our subjects according to clinical characteristics. We found that rs12794714 SNP was protector against asthma severity and asthma risk for smoker and men's subgroups. Furthermore, our results

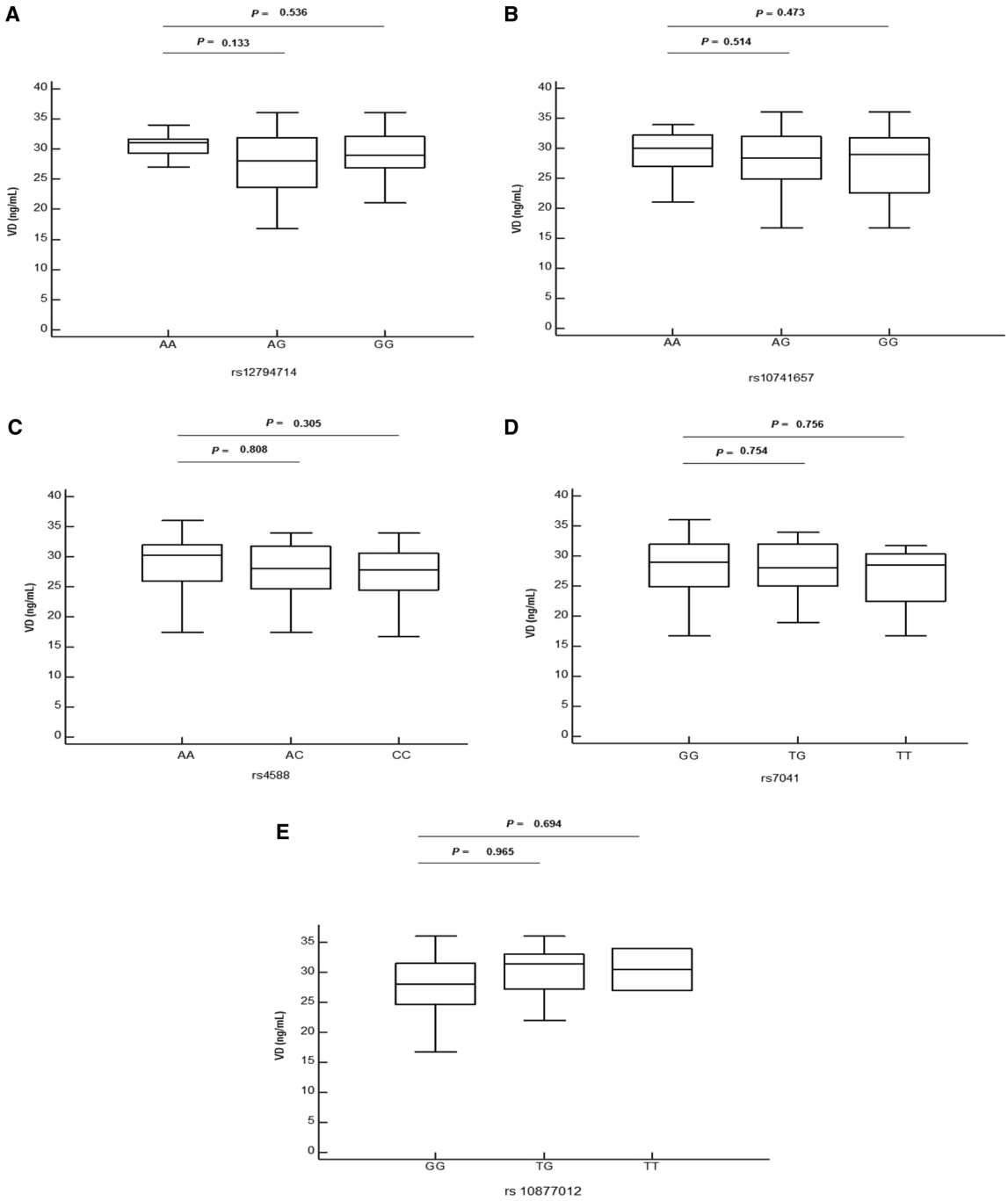

Fig. 3 The distribution of serum vitamin D levels among healthy controls according to VD gene metabolism pathway. The distribution of serum vitamin D levels among healthy controls according to **a** rs12794714 genotypes, **b** rs10741657 genotypes, **c** rs4588 genotypes, **d** rs7041 genotypes and **e** rs10877012 genotypes

show a very strong association between rs10877012 SNP and smoking habits, subjects' sex and age with promoting mild or moderate asthma development. We stratified our subjects according to different clinical, genetic and environmental factors. We also investigated the expression of total 25(OH)D serum concentration in our population in association with genetic and clinical parameters.

We noted that the mean total VD level in asthmatic patients was significantly lower than controls, which is in concordance with previous studies [17, 18]. Vitamin D metabolism is influenced not only by lack of exposure to sunlight or food sources of vitamin D, liver and kidney function, but also by individual's genetic identity. In COPD, Janssens et al. [19] found that the rs7041 SNP was

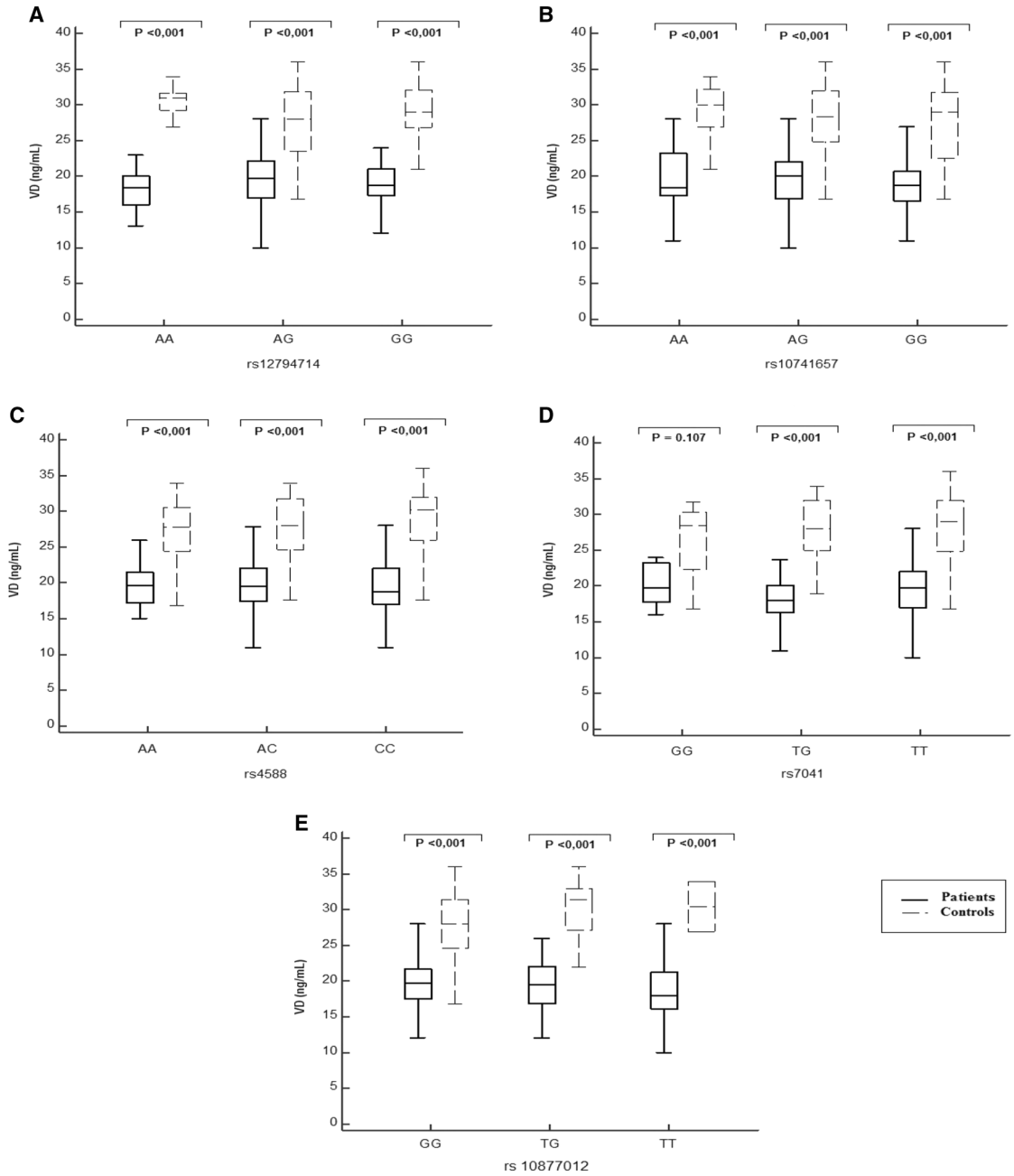

Fig. 4 The distribution of vitamin D levels between patients and healthy controls according to VD gene metabolism pathway. The distribution of serum vitamin D levels among patient and control groups according to: **a** rs12794714 genotypes, **b** rs10741657 genotypes, **c** rs4588 genotypes, **d** rs7041 genotypes and **e** rs10877012 genotypes

associated with low VD levels in Belgium patients. Other studies found that lower VD concentrations were strongly associated with both rs7041 and rs4588 SNPs in premenopausal , Hispanic and African American women [20, 21]. However, we did not observe a difference in vitamin D levels according to studied variants. In the same way, Jollife et al. [22, 23] and Batmaz et al [24] found that the VD status in asthmatic adults and children was not affected by genetic variants in GC and CYP2R1 genes.

Many epidemiological studies suggest that women are at increased risk of developing adult-onset of asthma and suffer from a more severe disease than men [25–27]. Our paper is the first study to demonstrate the association between subjects' sex and rs10877012 SNP with asthma risk. We revealed that women having the rs10877012 TT genotype were 6.70 times more likely to develop asthma in our population. Our results are in agreement with a case–control study of colorectal cancer (CRC) in

Northeast Chinese population; Gong et al [28] demonstrates that homozygote TT variant genotypes increased the risk of CRC significantly in women.

CYP27B1 encodes the enzyme that catalyzes the conversion of 25(OH)D to its active form, 1,25(OH)$_2$D. Variations in CYP27B1 may thus contribute to tissue availability of vitamin D, showing a high risk of type 1 diabetes, gestational diabetes mellitus and chronic hepatitis C [29–31]. Variations in this gene that reduce the efficiency of the hydroxylation of 25OHD to 1,25OH could lead to serum 25OHD concentrations appear normal or even high, but the concentration of the active ligand 1,25OHD may be suboptimal. We can suggest that 1.25OHD levels associated with deregulation in female hormone expression make women more susceptible to develop asthma. In fact, previous studies have shown that oestrogen can promote the formation of activated vitamin D by stimulating the secretion of parathyroid hormone [32, 33]. In our study, 82 were postmenopausal or on the brink of menopause (aged more than 50); thus, their oestrogen levels had declined significantly which affect the PTH level.

Obesity was known as a risk factor to develop many diseases as COPD and asthma [34–36]. In adults, the relationship between asthma and BMI has been consistently detected in women as mentioned in several studies [36–39]. Our findings revealed an association of CYP2R1 rs12794714 SNP with protection from asthma for overweight subjects, we also found it to be a protector against asthma severity. Data show that rs12794714 SNP was protector against colorectal cancer and chronic human virus infection (HBV) [40, 41]. However, other studies did not find any association between rs12794714 SNP and multiple sclerosis, type 1 diabetes and fatal prostate cancer [42–44].

The protective effect of the SNP until now is not clear and not studied. Robin et al. [45] demonstrated that the major allele of rs12794714 was associated with higher 25(OH)D concentrations while others did not find any association [46].

In our study, the presence of rs4588 was associated with higher risk for asthmatics who lived in the urban zone. Additionally, previous data have been shown a significant association of GC rs4588 SNP with lower vitamin D status [47–52]. Thus, an additive effect of the environmental factors (less sun exposure, humidity, air pollution) and genetic GC variant, leads probably to unbalance of Th1/Th2 and inflammations.

When we classified our subjects into asthmatics with or without atopy subgroups, we found that the same SNP rs4588 was a protector factor in particular atopy related to mites. Our findings are in contradiction with Bosse study, which concluded that there was no significant association between vitamin D level, atopy and asthma risk [53]. We hypothesized that hygiene, quality of life and socio-economic level can influence results in the same subgroup of asthmatics and between case–control subjects.

Conclusion

We observed a significant difference in vitamin D levels between asthmatic patients and controls but not with vitamin D gene pathway polymorphisms or other clinical factors studied. We found that rs12794714 SNP of CYP2R1 gene and rs10877012 SNP of CYP27B1 gene were associated with asthma risk. Further, not only the genetic factors are responsible of triangular relationship between SNPs, vitamin D level and asthma but also lifestyle, socio-economical and physiological factors may influence the vitamin D level so protect or not from diseases. However, the major limitation of the current study is the relatively small number of subjects which could have affected the statistical power of the results. Studies with higher number of subjects were needed.

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Compliance with Ethical Standards

Conflict of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Lung

Association between Vitamin D metabolism gene polymorphisms and risk of Tunisian adults 'asthma

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Supplemental table 1 Association between CYP2R1 rs12794714 SNP and risk of asthma according to Body Mass Index.

SNP rs12794714	BMI Classification	Genotypes	Population		OR (IC)	P
			Case (%)	Control (%)		
	Normal weight	GG	6 (31.57)	16 (34.78)	1	
		AG	11(57.89)	21(45.65)	1.38(0.41-4.85)	0.58
		AA	2(10.52)	9(19.56)	0.60(0.07-3.55)	0.57
		AA+AG	13(68.42)	30(65.21)	1.15(0.36-3.86)	0.80
	Over weight	GG	12(60)	17(30.90)	1	
		AG	7(35)	34(61.81)	**0.29(0.09-0.89)**	**0.02**
		AA	1(5)	4(7.27)	0.36(0.01-3.32)	0.37
		AA+AG	8(40)	38(69.09)	**0.30(0.10-0.88)**	**0.02**
	Obese	GG	16(40)	13(37.14)	1	
		AG	21(52.5)	16(45.71)	1.06(0.39-2.87)	0.89
		AA	3(7.5)	6(17.14)	0.41(0.07-2.01)	0.25
		AA+AG	24(60)	22(62.85)	0.88(0.34-2.28)	0.80

Supplemental table 2 Association between Age, Genotypes distribution of rs10877012 and risk of asthma

SNP			**Population**			
rs 10877012	**Age**	**Genotype**	**Case (%)**	**Control (%)**	**OR**	**P**
	[18-46]	GG	29(47.54)	62(64.58)	1	
		TG	22(36.06)	27(28.12)	1.73(0.84-3.57)	0.12
		TT	10(16.39)	7(7.29)	**3,01(1,03-9,20)**	**0.03**
		TG+TT	32(52.45)	34(35.41)	**2,00(1,04-3,88)**	**0.03**
	[47-71]	GG	44(48.88)	23(43.39)	1	
		TG	35(38.88)	24(45.28)	0.76(0.36-1.58)	0.46
		TT	11(12.22)	6(11.32)	0.95(0.31-3.12)	0.94
		TG+TT	46(51.11)	30(56.60)	0.80(0.40-1.59)	0.52

Supplemental table 3 Association between rs10741657, rs12794714 and rs10877012 SNPs and smoking habits with asthma risk

		Non Smoker		Active Smoker		OR1	P	OR2	P
SNPs	Genotypes	**CASE (%)**	**CONTROL (%)**	**CASE(%)**	**CONTROL (%)**				
rs10741657	GG	27(37.5)	31(39.24)	7(36.84)	6(16.66)	1		1	
	AG	30(41.66)	35(44.30)	7(36.84)	14(38.88)	0.98 (0.48-2.01)	0.96	0.43 (0.09-1.86)	0.24
	AA	15(20.83)	13(16.45)	5(26.31)	16(44.44)	1.32 (0.52-3.32)	0.54	0.27 (0.05-1.24)	0.07
	AG+AA	45(62.49)	48(60.75)	12(63.15)	30(83.32)	1.07 (0.55-2.08)	0.82	0.35 (0.09-1.29)	0.09
rs12794714	GG	30(43.47)	24(29.26)	11(57.89)	11(28.94)	1		1	
	AG	30(43.47)	49(59.75)	5(26.31)	21(55.26)	0.49 (0.24-0.99)	**0.04**	**0.24 (0.06-0.87)**	**0.02**
	AA	9(13.04)	9(10.97)	3(15.78)	6(15.78)	0.80 (0.26-2.39)	0.68	0.51 (0.08-2.61)	0.40
	AG+AA	39(56.51)	58(70.72)	8(42.09)	27(71.04)	0.54 (0.27-1.06)	**0.07**	**0.3 (0.09-0.96)**	**0.03**
rs10877012	GG	25(35.71)	48(58.53)	12(66.66)	19(50)	1		1	
	TG	26(37.14)	29(35.36)	5(27.77)	11(28.94)	1.71 (0.83-3.54)	0.13	0.72 (0.18-2.62)	0.61
	TT	19(27.14)	5(6.09)	1(5.55)	8(21.05)	**7.13 (2.46-23.70)**	0.0001	0.20 (0.008-1.51)	0.12
	TG+TT	45(64.28)	34(41.45)	6(33.32)	19(50)	**2.52 (1.31-4.92)**	0.005	0.50 (0.14-1.62)	0.24

OR1: Non-smoker Asthmatics vs Healthy Non-smoker

OR2: Active smoker Asthmatics vs Healthy smoker

Supplemental table 4 Association between Atopy and rs4588 SNP among Asthmatic subjects

SNP	Genotypes	**Mites Atopy (%)**	**non atopy (%)**	**Atopy (%)**	**OR1**	**P**	**OR2**	**P**
rs 4588	CC	12 (52.17)	8 (25)	23 (47.91)	1		1	
	AC	6 (26.08)	18 (56.25)	13 (27.08)	**0. 25(0.08-0.74)**	**0.01**	**0. 23(0.05-0.82)**	**0.02**
	AA	5 (21.73)	6 (18.75)	12 (25)	0.70 (0.19-2.62)	0.57	0.56 (0.11-2.59)	0.44
	AA+AC	11 (47.81)	24 (75)	25 (52.08)	**0. 36(0.13-0.96)**	**0.04**	**0. 31(0.09-0.98)**	**0.04**

OR1 asthmatics with atopy vs asthmatics with no-Atopy

OR2 asthmatics with Mites Atopy vs Asthmatics with no Atopy