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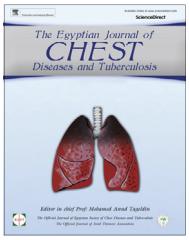

ORIGINAL ARTICLE

Association between vitamin D receptor gene FokI polymorphism and atopic childhood bronchial asthma

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KEYWORDS

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Abstract *Objective:* To investigate the association between vitamin D receptor (VDR) gene FokI polymorphism and atopic bronchial asthma in Egyptian children.

Material and methods: Polymerase chain reaction-restriction fragment length polymorphism was used to determine VDR gene FokI polymorphism in 180 asthmatic children (atopic, $n = 90$ and non-atopic, $n = 90$) and 180 age and gender matched healthy children. Serum levels of total IgE were measured by ELISA. Skin prick test was performed on all patients.

Results: The frequency of FF genotype was significantly higher in healthy children ($n = 90$, 50%) versus asthmatic children ($n = 39$, 21.7%, $\chi^2 = 5.852$, OR = 0.23, 95% CI = 0.07–0.78, $p = 0.016$). The frequency of FF genotype was significantly higher among healthy children (n = 90, 50%) compared to atopic asthmatic children ($n = 12$, 13.4%, $\chi^2 = 9.745$, OR = 0.11, 95% CI = 0.02–0.46, $p = 0.002$). Also, its frequency was significantly higher among non-atopic asthmatic children ($n = 27$, 30%) compared to atopic asthmatic children ($n = 12$, 13.4%, $\chi^2 = 4.394$, OR = 0.21, 95% CI = 0.04–0.94, $p = 0.041$). Total IgE differed significantly among the three VDR gene FokI polymorphic genotypes in children with atopic asthma ($p = 0.007$) and the highest median level was detected in ff genotype.

Conclusion: Our findings raise the susceptibility of VDR gene to be a candidate gene for atopic childhood bronchial asthma.

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Introduction

Vitamin D physiology has revealed that vitamin D is not merely a micronutrient which plays a role in calcium homeostasis but also a pluripotent hormone with immunomodulatory functions. It has also been found that the enzyme 1α-hydroxylase, which catalyzes the last and rate limiting step

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in the synthesis of active 1,25-dihydroxyvitamin D3, and the vitamin D receptor (VDR), which mediates the actions of vitamin D, are expressed widely in the body, including the lungs and cells of immune system [1].

Emerging studies have supported the role of vitamin D in asthma and allergy. In children with asthma, those with lower serum vitamin D have a higher risk of hospitalization [2,3], greater use of anti-inflammatory medications [2,4] and more airway hyper-responsiveness [2,3]. Moreover, researchers found that in children with asthma low serum vitamin D levels were associated with higher IgE [2,5].

VDR, encoded by the VDR gene located within the region q13–26 of chromosome 12, is a member of the nuclear receptor family subgroup NR1I [6]. Multiple studies have reported a significant association between polymorphisms in VDR gene and asthma as well as a range of autoimmune conditions, although the findings between cohorts have not been uniform [7–9]. However, no attempt was addressed to the question of whether any of VDR gene variants could affect total IgE level. Therefore, we selected a VDR gene FokI variant, which is known to have a specified extent for asthma and/or other pulmonary diseases [7,10], to evaluate its association with asthma in Egyptian children. Moreover, its relation to total IgE and the possibility of its role in atopic childhood asthma were studied.

Material and methods

This study included 180 asthmatic children (atopic, $n = 90$ and non-atopic, $n = 90$) recruited from the Pediatric Chest Clinic, Children's Hospital, Ain Shams University. They were diagnosed according to the clinical manifestations [11] and confirmed by spirometry with measurements of FEV_1 according to the standards of the European Respiratory Society and the American Thoracic Society [12]. Age and gender matched non-atopic, non-asthmatic healthy children ($n = 180$) were chosen as controls. They were selected from the geographic area surrounding the place of study. There was no significant difference in school grade between children participating in the study.

Skin prick tests (SPTs) were performed on all patients. Atopy was defined as at least one positive SPT result for common inhalant allergens (with positive histamine prick result). It was carried out using stainless-steeled lancets. The panel used was the Taiwan Asian panel (as it was found to be the most matching panel to Egyptians)that includes; House dust Mite Farinae, Mite pterony, Cockroach Mix, Feather Mix, Dogs' and Cats' dander, Candida, Aspergillus, Cladosporium ,Penicillium, Alternaria, Grass Mix, Bremuda Grass,Wheat, Cod fish, Pork, Beef, Egg, Whole, Egg Yolk, Milk, Yeast Brewer, Soybean, Peanut, Vegetable Mix, Rag wd Mix II, Pine Mix, Cotton wd, Mulberry Mi, Pigweed Mix, Corn, Crab, Shellfish Mix, Shrimp, and Eucalyptus [13,14]. Histamine solution (10 mg/ml) and glycerinated saline were used as positive and negative controls. SPTs were considered positive if there was a wheal of 3 mm in diameter or larger.

This study has complied with the principles laid down in the Declaration of Helsinki, adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964, and recently amended at the 64th World Medical Assembly, Fortaleza, Brazil, October 2013. The entire protocol was approved by institutional ethics committee. All parents or care givers provided signed informed consent for participation in the study as required.

Blood sample collection and processing

Five milliliter of venous blood sample was withdrawn from each participant under complete aseptic conditions and divided into two portions as follows: 2.0 ml of blood was placed in an EDTA containing tube for genotyping of VDR gene FokI polymorphism and the remaining 3.0 ml of blood was used for the separation of serum. The separated serum samples were kept frozen at −80 °C until used in the quantitative determination of total IgE according to the manufacturer's instructions of the human total IgE ELISA kit (General Biologicals Corporation, Taiwan).

Peripheral blood mononuclear cells (PBMCs) were separated from blood samples according to the manufacturer's instructions of Sigma Histopaque-1077 (Sigma–Aldrich, Inc., St. Louis, MO, USA). DNA was extracted from PBMCs using a QIAamp DNA blood mini kit (QIAGEN, Valencia, CA). The DNA purity and concentration were determined by spectrophotometric measurement of absorbance at 260 and 280 nm. Following DNA extraction from isolated PBMCs, a 200 µl sample yielded 6 µg of DNA (30 ng/µl) with an A260/A280 ratio of 1.7–1.9. Extracted DNA was stored at -80 °C until used.

Genotyping of VDR gene FokI polymorphism

The genotypes were determined by a standard Polymerase Chain Reaction-restriction fragment length polymorphism (PCR-RFLP) method.

Briefly, PCR reaction was performed in 25 µl 1xPCR buffer containing 20 pmol of each primer (Forward: 5′-CCTGGCA CTGACTCTGGCTCTG-3′ and Reverse: 5′-GGCTCCCTT CATGGAAACACCT-3′, GenBank: NT_029419.12), 2.5 mM $MgCl_2$, 200 mM of each dNTP, 50 ng DNA and 1.25 U Taq polymerase. Following initial denaturation at 94 °C for 5 min, amplification was performed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s and extension at 72 °C for 30 s. Final extension was allowed to proceed at 72 °C for 5 min. 5 µl of each PCR product was digested overnight with 1 U FokI restriction enzyme (MBI, Fermentas, Lithuania) at 37 °C. Electrophoresis was done through a 2% agarose gel containing ethidium bromide then the gel was visualized under UV illumination using gel documentation system (BioRad-Gel. 2000, Italy). By using this protocol, a 270 bp fragment encompassing the FokI polymorphic site was amplified. FokI digested the first ATG and yielded two products: 63 bp and 207 bp fragments (f allele), whereas the T to C transition destroyed the FokI site (F allele). Therefore, the homozygous FF genotype displayed only 270 bp fragment, ff homozygote displayed 63 bp and 207 bp fragments and Ff heterozygote displayed the three fragments.

Statistical analysis

The analysis was done using the Statistical Package for the Social Sciences (SPSS software version 19, SPSS Inc., Chicago, IL). Single nucleotide polymorphism (SNP) was assessed for genotypic association analysis among asthmatic and healthy

children. The genotype data of the tested SNP were then used to estimate Hardy–Weinberg equilibrium by comparing genotype frequencies within groups by a χ^2-test. Fisher's exact test was applied to analyze the comparison of the frequencies of discrete variables between asthmatic and healthy children. The odds ratios (ORs) with 95% confidence intervals (95% CI) were also calculated to estimate risk of asthma associated with the VDR gene FokI polymorphic genotypes. Using the Non-Parametric Kruskal–Wallis (χ^2) test, the mean rank and median of serum total IgE were estimated. Total IgE levels were also correlated with VDR gene FokI polymorphic genotypes. Statistical significance was set at a value of $p < 0.05$.

Results

The clinical characteristics of asthmatic and healthy children are shown in Table 1. The products of PCR-RFLP are shown in Figure 1. Genotype frequencies in asthmatic children ($p = 0.657$) and healthy children ($p = 0.171$) were consistent

Table 1 Clinical characteristics of asthmatic and healthy children.

Characteristics	Asthmatic children ($n = 180$)	Healthy children ($n = 180$)	P value
Age (years ± SD)	7.98 ± 3.22	8.5 ± 2.22	0.411
Total frequency of males (number, percent)	144 (80%)	144 (80%)	1.000
Total frequency of females (number, percent)	36 (20%)	36 (20%)	1.000
BMI (mean ± SD)	21.9 ± 4.0	17.6 ± 1.7	**< 0.001****
FEV_1 (% of predicted ± SD)	74.5 ± 12.5	88.9 ± 8.2	**< 0.001****
Passive smoking (number, percent)			
Positive	90 (50%)	54 (30%)	0.12
Negative	90 (50%)	126 (70%)	
Family history of asthma (number, percent)			
Positive	90 (50%)	0 (0%)	**< 0.001****
Negative	90 (50%)	180 (100%)	

** $p < 0.01$ is highly significant.

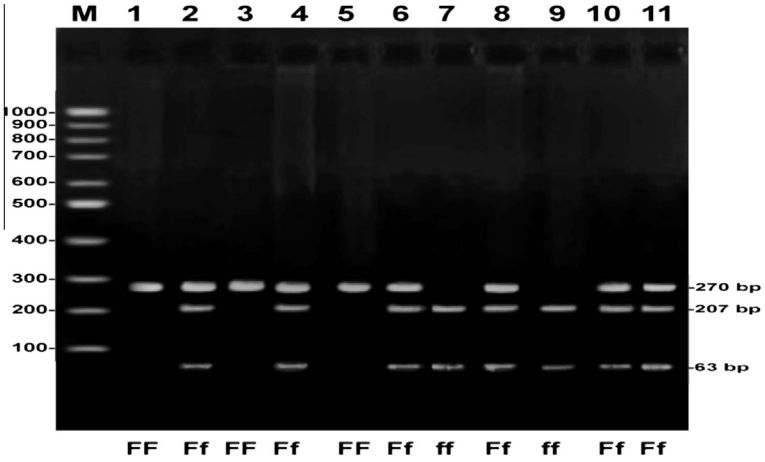

Figure 1 Representative agarose gel electrophoresis for detection of FokI VDR polymorphism. Lane (M): 100 bp molecular weight marker, lanes 1, 3 and 5: homozygous FF genotype; lanes 2, 4, 6, 8, 10, 11: heterozygous Ff genotype and lanes 7 and 9 homozygous ff genotype.

Table 2 Genotype distribution of FokI VDR polymorphism between asthmatic and healthy children.

Genotype	Asthmatic children ($n = 180$)	Healthy children ($n = 180$)	OR (95% CI)	χ^2	P value
FF	39 (21.7%)	90 (50%)	0.23 (0.07–0.78)	5.852	0.016*
Ff	84 (46.7%)	60 (33.3%)	0.74 (0.22–2.49)	0.241	0.431
ff	57 (31.6%)	30 (16.7%)	1 (Ref.)[a]		

All groups were in Hardy–Weinberg equilibrium ($p > 0.5$).
[a] ff genotype as reference (OR = 1.0).
* $p < 0.05$ is significant.

Table 3 Genotype distribution of FokI VDR polymorphism between study groups.

Genotype	Children with atopic asthma ($n = 90$)	Healthy children ($n = 180$)	OR (95% CI)	χ^2	*P* value	Children with non-atopic asthma ($n = 90$)	Healthy children ($n = 180$)	OR (95% CI)	χ^2	*P* value	Children with atopic asthma ($n = 90$)	Children with non-atopic asthma ($n = 90$)	OR (95% CI)	χ^2	*P* value
FF	12 (13.4%)	90 (50%)	0.11 (0.02–0.46)	9.745	**0.002****	27 (30%)	90 (50%)	0.50 (0.12–2.12)	0.895	0.281	12 (13.4%)	27 (30%)	0.21 (0.04–0.94)	4.394	**0.041***
Ff	39 (43.3%)	60 (33.3%)	0.50 (0.13–1.87)	1.073	0.24	45 (50%)	60 (33.3%)	1.25 (0.30–5.23)	0.094	0.521	39 (43.3%)	45 (50%)	0.40 (0.12–1.35)	2.215	0.117
ff	39 (43.3%)	30 (16.7%)	1 (Ref.)[a]			18 (20%)	30 (16.7%)	1 (Ref.)[a]			39 (43.3%)	18 (20%)	1 (Ref.)[a]		

[a] ff genotype as reference (OR = 1.0).
* $p < 0.05$ is significant.
** $p < 0.01$ is highly significant.

with Hardy–Weinberg equilibrium. The frequency of FF genotype was significantly higher in healthy children ($n = 90$, 50%) versus asthmatic children ($n = 39$, 21.7%, $\chi^2 = 5.852$, OR = 0.23, 95% CI = 0.07–0.78, $p = 0.016$), Table 2. The frequency of FF genotype was significantly higher among healthy children ($n = 90$, 50%) compared to atopic asthmatic children ($n = 12$, 13.4%, $\chi^2 = 9.745$, OR = 0.11, 95% CI = 0.02–0.46, $p = 0.002$). Also, its frequency was significantly higher among non-atopic asthmatic children ($n = 27$, 30%) compared to atopic asthmatic children ($n = 12$, 13.4%, $\chi^2 = 4.394$, OR = 0.21, 95% CI = 0.04–0.94, $p = 0.041$), Table 3.

The mean rank and median of total IgE were significantly higher in atopic asthmatic children ($p < 0.001$) compared to healthy and non-atopic asthmatic children, Table 4. Furthermore, total IgE differed significantly among the three VDR gene FokI polymorphic genotypes in children with atopic asthma ($p = 0.007$) and the highest median level was detected in ff genotype, (Table 5).

Discussion

This is the first report that demonstrates a significant association between the FokI VDR gene polymorphism and the risk of atopic childhood asthma in Egyptian children. It was specifically noticed that children carrying FF genotype had a significantly lower prevalence of atopic asthma. These findings represent another evidence for the role of vitamin D in childhood asthma.

Our findings are supported by several clinical and experimental observations. Researchers found that vitamin D insufficiency is common in children with mild to moderate persistent asthma and is associated with higher odds of severe exacerbation [3]. Epidemiologic studies have shown that maternal vitamin D intake during pregnancy protects from childhood wheezing [15,16]. It has also been reported that children whose mothers had high vitamin D levels in pregnancy had an increased risk of eczema and asthma, suggesting that the time point of vitamin D supplementation seems to determine the susceptibility to atopic disease [17]. Additionally, it has been found that the FF genotype of VDR gene FokI polymorphism was associated with increased transcriptional activity of VDR [18–21]. On experimental level, in a murine asthma model, the VDR was found to be necessary for the development of an allergic airway inflammation [22].

Several studies classified VDR gene as one of the candidate genes of asthma [7–8,23]. To our knowledge, no attempt was addressed to the question of whether any of VDR gene variants could act as a protective or predictive variant for atopic asthma. Since genotype frequencies of genes may vary according to population and ethnicity, we conducted this study to determine genotype frequencies of VDR gene FokI polymorphism in Egyptian asthmatic children. The relation between this variant and the level of total IgE was evaluated. Also, positive skin prick test was done to confirm the diagnosis of atopic asthma.

In this study, the frequency of FF genotype was significantly higher among healthy children and non-atopic asthmatic children compared to atopic asthmatic children. This was in agreement with a recent study which detected significant difference in the distribution of FokI genotype frequencies be-

Table 4 Comparison between asthmatic and healthy children regarding serum total IgE.

Total IgE (IU/μl)	Children with atopic asthma (n = 90)	Children with non-atopic asthma (n = 90)	Healthy children (n = 180)	Statistics
Median (range)	344.5 (200–400)	99.5 (59–115)	64.5 (43–95)	$p < 0.001$**
Mean Rank	75.5	39.68	21.32	$\chi^2 = 23.42$
95% CI	251.7–365.8	84.3–105.8	55–83.5	

** $p < 0.01$ is highly significant.

Table 5 Relation between median levels of total IgE (IU/μl) and genotypes of FokI VDR polymorphism in different groups of the study.

Groups	Total IgE (IU/μl)	Genotypes			Statistics
		FF	Ff	ff	
Children with atopic asthma	Median (range)	218.5 (201–260)	250 (200–395)	360 (300–400)	p = **0.007****
	Mean Rank	6.0	13.31	20.62	χ^2 = **9.871**
	95% CI	205.5–255	222.6–340.5	344.8–391.9	
Children with non-atopic asthma	Median (range)	89 (59–112)	98 (60–115)	105.5 (99–112)	$p = 0.172$
	Mean Rank	12.78	14.83	21.25	$\chi^2 = 3.519$
	95% CI	60.4–109.3	66.3–107.5	99.6–111.6	
Healthy children	Median (range)	64 (49–93)	71.5 (43–95)	65 (47–85)	$p = 0.988$
	Mean Rank	15.70	15.45	15.0	$\chi^2 = 0.024$
	95% CI	51.3–85.4	44.5–92.6	48–84	

** $p < 0.01$ is highly significant.

tween asthmatic and healthy children [24]. Indeed, another study did not find any relation between genotypes of FokI VDR polymorphisms and asthma [25].

In an attempt to evaluate the association of VDR genetic variants and susceptibility to atopy, researchers found that TaqI, ApaI and BsmI VDR SNPs have been associated with asthma and atopy [7]. In this study, it was not surprising that median level of total IgE was significantly higher in children with atopic asthma compared to children with non-atopic asthma and healthy controls. This result confirmed the usefulness of total IgE as indicator of atopic diseases [26,27]. Furthermore, we found that in children with atopic asthma the homozygote ff genotype has been associated with the highest median level of total IgE, while the homozygote FF genotype has been associated with the lowest median level of total IgE. The data we found do suggest that FF genotype could play a protective role against atopic childhood asthma.

In conclusion, the elucidation of the precise role of VDR polymorphism in the pathogenesis of atopic childhood asthma has the potential to have profound effects on the ability to prevent and treat this disorder. In this study, we have reported a novel association between the FokI VDR gene polymorphism and the risk of atopic childhood asthma. Further studies on a large cohort of patients will be necessary to confirm these findings. Along with other known asthma risk genes, the addition of VDR involvement in the understanding of asthma/atopy pathogenesis will shed light for better control and treatment.

Conflict of interest

None.

References

[1] S. Hansdottir, M. Monick, Vitam. Horm. 86 (2011) 217–237.

[2] J.M. Brehm, J.C. Celedón, M.E. Soto-Quiros, L. Avila, G.M. Hunninghake, E. Forno, D. Laskey, J.S. Sylvia, B.W. Hollis, S.T. Weiss, A.A. Litonjua, Am. J. Respir. Crit. Care Med. 179 (2009) 765–771.

[3] J.M. Brehm, B. Schuemann, A.L. Fuhlbrigge, B.W. Hollis, R.C. Strunk, R.S. Zeiger, S.T. Weiss, A.A. Litonjua, J. Allergy Clin. Immunol. 126 (2010) 52–58.

[4] D.A. Searing, Y. Zhang, J.R. Murphy, P.J. Hauk, E. Goleva, D.Y. Leung, J. Allergy Clin. Immunol. 125 (2010) 995–1000.

[5] S. Bose, P.N. Breysse, M.C. McCormack, N.N. Hansel, R.R. Rusher, E. Matsui, R. Peng, J. Curtin-Brosnan, G.B. Diette, For the center for childhood asthma in the urban environment, Nutr. J. 12 (2013) 81.

[6] N. Rochel, D. Moras, Curr. Top. Med. Chem. 6 (2006) 1229–1241.

[7] A.H. Poon, C. Laprise, M. Lemire, A. Montpetit, D. Sinnett, E. Schurr, T.J. Hudson, Am. J. Respir. Crit. Care Med. 170 (2004) 967–973.

[8] B.A. Raby, R. Lazarus, E.K. Silverman, S. Lake, C. Lange, M. Wjst, S. Weiss, Am. J. Respir. Crit. Care Med. 170 (2004) 1057–1065.

[9] C. Vollmert, T. Illig, J. Altmüller, S. Klugbauer, S. Loesgen, L. Dumitrescu, M. Wjst, Clin. Exp. Allergy 34 (2004) 1841–1850.

[10] W. Liu, W.C. Cao, C.Y. Zhang, L. Tian, X.M. Wu, J.D. Habbema, Q.M. Zhao, P.H. Zhang, Z.T. Xin, C.Z. Li, H. Yang, Int. J. Tuberc. Lung Dis. 8 (2004) 428–434.

[11] Global Initiative for Asthma [GINA]. GINA Report, Global Strategy for Asthma Management and Prevention, 2008.

[12] M.R. Miller, J. Hankinson, V. Brusasco, F. Burgos, R. Casaburi, A. Coates, R. Crapo, P. Enright, C.P.M. van der Grinten, P. Gustafsson, R. Jensen, D.C. Johnson, N. MacIntyre,

R. McKay, D. Navajas, O.F. Pedersen, R. Pellegrino, G. Viegi, J. Wanger, Eur. Respir. J. 26 (2005) 319–338.
[13] O.A. Hamid, S. Elfedawy, S.K. Mohamed, H. Mosaad, Eur. Arch. Otorhinolaryngol. 266 (2009) 1569–1573.
[14] M.M. Morcos, W.M. Morcos, M.A. Ibrahim, M.A. Shaheen, Minerva Pediatr. 63 (2011) 19–26.
[15] C.A. Camargo Jr., S.L. Rifas-Shiman, A.A. Litonjua, J.W. Rich-Edwards, S.T. Weiss, D.R. Gold, K. Kleinman, M.W. Gillman, Am. J. Clin. Nutr. 85 (2007) 788–795.
[16] G. Devereux, A.A. Litonjua, S.W. Turner, L.C. Craig, G. McNeill, S. Martindale, P.J. Helms, A. Seaton, S.T. Weiss, Am. J. Clin. Nutr. 85 (2007) 853–859.
[17] C.R. Gale, S.M. Robinson, N.C. Harvey, M.K. Javaid, B. Jiang, C.N. Martyn, K.M. Godfrey, C. Cooper, Eur. J. Clin. Nutr. 62 (2008) 68–77.
[18] H. Arai, K. Miyamoto, Y. Taketani, H. Yanamoto, Y. Iemori, K. Morita, T. Tonai, T. Nishisho, S. Mori, E. Takeda, J. Bone Miner. Res. 12 (1997) 915–921.
[19] E.M. Colin, A.E. Weel, A.G. Uitterlinden, C.J. Buurman, J.C. Birkenhager, H.A. Pols, J.P. van Leeuwen, Clin. Endocrinol. (Oxf) 52 (2000) 211–216.
[20] P.W. Jurutka, L.S. Remus, G.K. Whitfield, P.D. Thompson, J.C. Hsieh, H. Zitzer, P. Tavakkoli, M.A. Galligan, H.T. Dang, C.A. Haussler, M.R. Haussler, Mol. Endocrinol. 14 (2000) 401–420.
[21] B.W. Ogunkolade, B.J. Boucher, J.M. Prahl, S.A. Bustin, J.M. Burrin, K. Noonan, B.V. North, N. Mannan, M.F. McDermott, H.F. DeLuca, G.A. Hitman, Diabetes 51 (2002) 2294–2300.
[22] A. Wittke, V. Weaver, B.D. Mahon, A. August, M.T. Cantorna, J. Immunol. 173 (2004) 3432–3436.
[23] A. Saadi, G. Gao, H. Li, C. Wei, Y. Gong, Q. Liu, BMC. Med. Genetics 10 (2009) 71.
[24] H. Maalmi, F.H. Sassi, A. Berraies, J. Ammar, K. Hamzaoui, A. Hamzaoui, Hum. Immunol. 74 (2013) 234–240.
[25] W.L. Fang, L.B. Gao, W.B. Liang, H. Xue, P. Bai, M.L. Lv, Y.Y. Wang, B. Zhou, L. Zhang, Iran. J. Allergy Asthma Immunol. 8 (2009) 141–147.
[26] P.J. Barnes, Clin. Exp. Allergy 39 (2009) 1145–1151.
[27] P.J. Gergen, S.J. Arbes Jr., A. Calatroni, H.E. Mitchell, D.C. Zeldin, J. Allergy Clin. Immunol. 124 (2009) 447–453.