



Toll-like receptor 2 and Toll-like receptor 4 polymorphisms and susceptibility to asthma and allergic rhinitis: A case–control analysis

Yousri M. Hussein^a, Hanan A. Awad^a, Sally M. Shalaby^{a,*}, Al-Shaymaa A. Ali^b, Saad S. Alzahrani^c

^a Medical Biochemistry Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt

^b Pediatric Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt

^c Family Medicine Department, Faculty of Medical Technology, Eltaief University, KAS

ARTICLE INFO

Article history:

Received 9 September 2011

Accepted 1 February 2012

Available online 17 February 2012

Keywords:

Asthma

Allergic rhinitis

Gene–environment interaction

Innate immunity

TLRs

ABSTRACT

The aim of the study was to investigate whether polymorphisms in genes encoding Toll-like receptors (TLR2 and TLR4) may modify relative risk for development of asthma or allergic rhinitis. The results showed that the genotype and allele frequencies of the TLR2 Arg753Gln and TLR4 Asp299Gly polymorphisms were not significantly different between asthmatic children or allergic rhinitis when compared to controls ($p > 0.05$ for each) or even when compared further with IgE level. However, it was shown that the mutant allele of TLR2 or TLR4 polymorphisms were significantly associated with the moderate–severe group compared to the mild group in both atopic asthmatics and allergic rhinitis group ($p > 0.001$ for each). In conclusion, our study demonstrates a lack of association of TLR2 and TLR4 polymorphisms with asthma and allergic rhinitis but suggests significant association between these genetic variants and the disease severity.

© 2012 Elsevier Inc. All rights reserved.

1. Introduction

Asthma as chronic inflammatory airway disease is considered to be the most common chronic disease and the leading cause of hospitalization in schoolchildren [1,2]. Allergic rhinitis is an inflammatory disorder affecting 10–25% of the population world wide with both local inflammation in the nose, and a systemic component that influences peripheral blood, bone marrow and lungs [3].

The development of asthma and allergic rhinitis results when a genetically susceptible individual is exposed to specific allergens. Allergy is believed to be related to imbalance in the T helper1 (Th1)/Th2 immune response and cytokine release, as well as allergen-specific immunoglobulin production [4].

Toll-like receptors (TLRs) recognize microbial patterns and play a crucial role in linking innate and adaptive immunity inducing a proinflammatory immune response that may counterbalance allergic diathesis. They initiate intracellular signaling pathways, bind to downstream protein kinases, induce activation of transcription factors, and initiate transcription of inflammatory cytokines and other host response elements [5]. It has been suggested that their ligands induce a shift towards Th1-dominated immunity that may coun-

terbalance the Th2 predominance in asthma and allergic rhinitis [6]. There are at least 10 known mammalian TLR family members, TLR2 and TLR4 are known to have a broad specificity for the recognition of microbial patterns. TLR2 is a key component in linking microbial products, apoptosis and host defense mechanisms [7]. It recognizes gram positive bacteria, mycobacterial species, protozoan parasites, microbial lipoproteins, glycoproteins, glycolipids, and nonenteric lipopolysaccharide (LPS) [8,9]. TLR4 recognizes LPS, part of the cell wall of gram-negative bacteria. Upon ligand binding cells stimulate the nuclear factor B pathway increasing cytokine formation and stimulate Th1 cytokine production in a variety of in vivo and in vitro models mediating the protection from allergic diseases [10].

TLRs polymorphisms may play role in the balance between Th1 and Th2 responses, and increased susceptibility to allergic airway diseases. In a study to quantify messenger RNA (mRNA) and protein expression of TLR2, TLR3, and TLR4 in the nasal mucosa of patients with seasonal allergic rhinitis before and after challenge with relevant pollens identified, protein expression for all three TLRs was demonstrated to increase in patients with rhinitis after challenge. This study raises the possibility that TLRs polymorphisms may have a role in the development of allergic airway inflammation [11].

Although some researches have studied the association between polymorphisms in the TLR2 and TLR4 in asthma and allergic rhinitis; but, the results are still heterogeneous [12–14]. Previous study reported the association of TLR2 Arg753Gln (+2408G>A)

Abbreviations: Th1, T helper1; TLRs, Toll-like receptors; LPS, lipopolysaccharide; IgE, immunoglobulin E.

* Corresponding author.

E-mail addresses: yousrihussein@hotmail.com (Y.M. Hussein), sallyshalaby@hotmail.com (S.M. Shalaby).

with higher levels of total serum immunoglobulin E (IgE) in atopic dermatitis [15]. Wernar et al. [16] suggested that TLR4 Asp299Gly (+986A > G) polymorphism modified response to endotoxin, which is an important trigger of asthma.

Our aim was to study the potential associations between polymorphisms in TLR2 Arg753Gln, and TLR4 Asp299Gly genes and the risk of asthma and allergic rhinitis in Egyptian children. Moreover, we determined the association of these polymorphisms on asthma and allergic rhinitis phenotypes including total IgE, and disease severity in asthmatic children and allergic rhinitis cases.

2. Patients and methods

The study was conducted by the Medical Biochemistry and Pediatric Departments at the Faculty of Medicine of Zagazig University – Egypt.

2.1. Participants

Two hundred fifty atopic children with asthma, 250 non-atopic children with asthma, and 150 children with allergic rhinitis were enrolled in this study.

Following the American Thoracic Society guidelines [17], asthma was confirmed by a history of chest tightness and wheezing during the previous 12 months, a greater than 12% reversibility of forced expiratory volume (FEV₁) spontaneously or after β_2 -agonist inhalation, and/or a methacholine provocation test result with a PC₂₀ less than 16 mg/ml. Atopy was defined by a positive skin prick test (wheal diameter $\geq$ 3 mm) to at least one of 12 common aeroallergens. Clinical phenotype was based on a questionnaire completed separately for each child by the parents. The questionnaire included demographics, medical history, skin-prick data, documentation of laboratory samples taken, and information on medicines taken during the last 12 months. The diagnosis and classification of the clinical severity of asthma was made according to the GINA guidelines [18]. The asthmatic children were categorized into severity groups by using level of FEV₁% predicted. The patients were classified as having mild (FEV₁ > 80%), moderate (FEV₁ 60–80%), or severe (FEV₁ < 60%). The diagnosis of allergic rhinitis and classification of the clinical severity was made according to Rhinitis scoring system [19]. None of the participants received antihistamine, systemic or topical corticosteroids in the 3 weeks prior to clinical evaluation and they all underwent skin prick testing.

2.2. Controls

The control group consisted of 250 children presented to the pediatric department of the Hospital of Zagazig University with other than respiratory or atopic complaints. The non-atopic control subjects had no history of asthma or other allergic diseases, negative skin prick tests, normal total IgE values ($\leq$ 100 IU/ml), normal

lung function tests, and no airway hyper-responsiveness (PC₂₀ > 25 mg/ml). Patients and controls belonged to the same ethnic group: African Caucasian. The study was approved by ethical committee of Faculty of Medicine, Zagazig University and written informed consent was obtained from all participants. Characteristics of the participants are presented in Table 1.

2.3. Total IgE measurements

Total serum IgE levels were measured by sandwich ELISA (Monobind Inc., Costa Mesa, California, USA), with an assay sensitivity for total IgE of 1.0 IU/mL.

2.4. Genotyping

Genomic DNA was extracted from whole blood using a genomic DNA purification kit (Promega, Madison, Wisconsin, USA). Participants were genotyped for the SNP of TLR2 Arg753Gln and TLR4 Asp299Gly in genomic DNA.

PCR was performed in a final volume of 25 μ L containing 3.5 μ L of H₂O, 5 μ L of genomic DNA, 1 μ L of each primer (1 μ M), and a 2 $\times$ Super Hot PCR Master Mix (12.5 μ L) (Bioron, Ludwigshafen am Rhein, Germany). The amplification was carried out using a PTC-100 thermal cycler (MJ Research, Inc., Watertown, Massachusetts, USA).

TLR2 Arg753Gln polymorphism was conducted by a tetra-primer amplification refractory mutation system–polymerase chain reaction (ARMS–PCR) [20]. The nucleotide sequences of the primers were: TLR2-F 5'-TATGGTCCAGGAGCTGGAGA, TLR2-R 5'-TGACATAAAGATCCCACTAGAC, TLR2-G 5'-GGTCTTGGTGTTCATTATCTTCC, and TLR2-A 5'-GGTCTTGGTGTTCATTATCTT-CT. The primers TLR2-F, TLR2-R, TLR2-G were used for detection of G allele and TLR2-F, TLR2-R, and TLR2-A were used for detection of A allele of TLR2 Arg753Gln. The 430-bp common product was amplified using TLR2-F and TLR2-R primers, and the 307-bp product was amplified using TLR2-F and TLR2-G, or TLR2-F and TLR2-A primers. The cyclic condition consisted of an initial denaturation step at 94 °C for 5 min, followed by 30 amplification cycles of denaturation for 45 s at 94 °C, annealing for 45 s at 62 °C, extension for 45 s at 72 °C, and one elongation step at 72 °C for 7 min.

Participants were genotyped for the TLR4 Asp299Gly polymorphism by multiplex allele-specific PCR [21]. Genotyping of the TLR4 Asp299Gly polymorphism was performed by amplification of genomic DNA using the following primers: outer upper primer 5'-CCTGAACCCATGAACCTTATCC-3', outer lower primer 5'-GTTAACTAATCTAAATGTTGCCATC-3', inner upper primer (Asp allele) 5'-GCATACTTAGACTACTACCTCGAaGA-3', and inner lower primer (Gly allele) 5'-GTCAAACAATTAATAAGTCAATAAAAC-3'. Cycling conditions were 95 °C 5 min; then nine cycles of 94 °C 30 s, X °C 30 s (where X is initially 72 °C, decreasing 1 °C per cycle to 64 °C), 72 °C 30 s; then 31 cycles of 94 °C 30 s, 64 °C 30 s, and

Table 1
Clinical profiles of study subjects.

	Asthma group N = 500	Allergic rhinitis group N = 150	Control group N = 150
Age (years)*	8.4 $\pm$ 2.6	8.3 $\pm$ 2.5	10.1 $\pm$ 2.3
Male/female	270/230	80/70	77/73
BMI (kg/m ²)	18.6 $\pm$ 3	18.5 $\pm$ 3.1	18 $\pm$ 2.8
Mean age at the onset of disease (years)†	4.2	4.5	
FEV ₁ (%)	85.4 $\pm$ 19	93.4 $\pm$ 15	96.5 $\pm$ 16
Log PC ₂₀ (mg/mL)*	0.35 $\pm$ 0.6	1.42 $\pm$ 0.2	1.35 $\pm$ 0.3
Log IgE (IU/mL)*	2.41 $\pm$ 0.5	2.24 $\pm$ 0.4	1.45 $\pm$ 0.6

* $P < 0.05$ for differences among the three groups.

† $p < 0.05$ for difference between asthma and allergic rhinitis groups.

72 °C 30 s; and finally 72 °C 10 min. The products for the reaction were allele-specific PCR products (Asp 147 bp, Gly 292 bp) and a constant product (385 bp). All the amplified products were analyzed on 2% agarose gels stained with ethidium bromide.

2.5. Statistical analysis

The results for continuous variables are expressed as means $\pm$ SD. Quantitative measurements of IgE were normalized by using a log transformation. The means of the groups were compared by ANOVA-test. The statistical significances of differences in frequencies of variants between the groups were tested using the chi-square (χ^2) test. In addition, odds ratios (ORs) and 95% CIs were calculated as a measure of the association of the TLRs alleles with asthma and allergic rhinitis. Genotype frequencies in cases and controls were tested for Hardy–Weinberg equilibrium, and any deviation between the observed and expected frequencies was tested for significance using the χ^2 test. A difference was considered significant if p was <0.05 . Data were analyzed using SPSS software version 11 (SPSS Inc., Chicago, Illinois, USA).

3. Results

The genotype and allele distribution for the TLR2 Arg753Gln and TLR4 Asp299Gly polymorphisms by study group was shown in Table 2. The genotype frequencies of the two SNPs were in agreement with Hardy–Weinberg equilibrium in all groups. Because the genotype AA of the TLR2 and the genotype GG of the TLR4 were rare, we combined GA and AA genotypes for TLR2 also AG and GG genotypes for TLR4 when calculating p values. The frequencies of the AA genotype of TLR2 polymorphism were 0.8% in atopic asthma patients, 0.4% in non-atopic asthma patients and

none in each allergic rhinitis and control groups. The frequencies of GG genotype of TLR4 SNP were 0.8% in atopic asthma, 0.4% in controls and no patient in non-atopic and allergic rhinitis groups was homozygous for the mutant allele. The genotype and allele frequencies of the TLR2 Arg753Gln and TLR4 Asp299Gly polymorphisms were not significantly different between atopic asthmatic children and non-atopic asthmatic children when compared to controls. When we compared between asthmatic children group as a whole with controls, again there was no significant difference of genotype and allele frequencies between children with asthma and control group ($p = 0.94$ for TLR2 and $p = 0.49$ for TLR4). Comparing these frequencies in atopic and non-atopic asthmatics, we found no difference and p values were not significant ($p = 0.45$ for TLR2 SNP and $p = 0.36$ for TLR4 SNP).

Concerning allergic rhinitis, the genotype and allele frequencies of the TLR2 and TLR4 polymorphisms were not significantly different between allergic rhinitis children and controls ($p = 0.72$ for TLR2 SNP and $p = 0.63$ for TLR4 SNP).

Next we studied the association of TLR2 Arg753Gln and TLR4 Asp299Gly polymorphisms with total IgE. We found that there was no effect of different TLR2 and TLR4 genotypes on serum IgE levels in all studied groups (Table 3).

Because only 15 patients were in severe asthma group, patients with moderate and severe asthma were combined into a single group for analysis of severity. It was observed that hetero- or homozygosity for the mutant allele of TLR2 or TLR4 polymorphisms was significantly associated with the moderate–severe group compared to the mild group in both atopic asthmatics and allergic rhinitis group whereas no difference was observed in the non-atopic asthmatics (Table 4). It was noticed that there was no patient in the mild group who was homozygous for the abnormal alleles of TLR2 and TLR4 polymorphisms in all patients groups.

Table 2
Genotype and allele frequencies of TLR2 and TLR4 polymorphisms in groups studied.

	Controls	Asthma group p^*	Atopic asthma p^*	Non-atopic asthma p^*	Allergic rhinitis p^*
TLR2 (Arg753Gln) Genotype					
GG	243	484	240	244	144
GA + AA	7	16	10	6	6
Allele					
G	493	968	488	493	294
A	7	19	12	7	6
TLR4 (Asp299Gly) Genotype					
AA	223	434	213	221	130
AG + GG	28	66	37	29	20
Allele					
A	473	932	461	471	280
G	29	68	39	29	20

* p calculated by chi square test as compared to controls As compared to controls.

Table 3
Effect of TLR2 and TLR4 gene polymorphisms on serum IgE levels in all studied groups.

		11 [†]	12 + 22	p^*
TLR2(Arg753Gln)	Atopic asthma	2.35 $\pm$ 0.45	2.37 $\pm$ 0.20	0.89
	Non-atopic asthma	1.50 $\pm$ 0.62	1.48 $\pm$ 0.22	0.94
	Allergic rhinitis	2.24 $\pm$ 0.45	2.19 $\pm$ 0.36	0.79
	Control group	1.43 $\pm$ 0.50	1.45 $\pm$ 0.30	0.92
TLR4(Asp299Gly)	Atopic asthma	2.41 $\pm$ 0.32	2.38 $\pm$ 0.22	0.59
	Non-atopic asthma	1.49 $\pm$ 0.51	1.51 $\pm$ 0.36	0.84
	Allergic rhinitis	2.20 $\pm$ 0.34	2.25 $\pm$ 0.26	0.53
	Control group	1.42 $\pm$ 0.40	1.46 $\pm$ 0.32	0.61

* Calculated by t -test.

[†] The major alleles are referred to as allele 1 and the minor alleles as allele 2.

Table 4

Relation between genotype distribution of TLR2 and TLR4 polymorphisms and disease severity.

	Atopic asthmatics			Nonatopic asthmatics			Allergic rhinitis		
	Mild	Moderate-severe		Mild	Moderate-severe		Mild	Moderate-severe	
	N = 180	N = 70	p	N = 142	N = 108	p	N = 95	N = 55	p
<i>TLR2(Arg753Gln)</i>									
GG	180	60	<0.001	140	104	0.41	95	49	0.001
GA	0	8		2	3		0	6	
AA	0	2		0	1		0	0	
<i>TLR4(Asp299Gly)</i>									
AA	175	38	<0.001	129	92	0.17	93	37	<0.001
AG	5	30		13	16		2	18	
GG	0	2		0	0		0	0	

For TLR2, GG group was compared with GA and AA combined and for TLR4, AA group was compared with AG and GG combined.

4. Discussion

Development of allergic airway inflammation is related to Th2 lymphocytes and their production of inflammatory cytokines as a part of the adaptive immunity, but recent data suggest that the response to innate immune stimuli seems to be critical to conditioning adaptive immunity. Accordingly, the microbial stimulation is important for the postnatal maturation of the immune system and induction of a skewing from atopic Th2 responses to Th1-dominated responses through stimulation of the innate immune system [22]. Because TLRs are involved in the recognition of the microbial environment and are capable of activation of innate immunity and regulation of adaptive immunity, TLRs polymorphisms may alter the capability to recognize microbes, leading to inadequate immune responses and increasing the susceptibility for inflammatory airway diseases [23].

Activation of TLR2 initiated a Th2 immune response in experimental asthma, thus supporting the hypothesis that TLR2 might play a critical role in asthma [24]. The polymorphism, TLR2 (+2408G>A) (rs5743708), is located within the intracellular portion of the receptor resulting in an amino acid exchange, Arg753 to Gln. When we compared the TLR2 Arg753Gln polymorphism between asthmatic group with controls, there was no significant difference of genotype and allele frequencies between both groups. Further asthmatic children were stratified into: atopic asthmatic and non-atopic asthmatic children. The genotype frequencies were not different with regard to the presence of atopy for TLR2 polymorphism. Our results were consistent with a study conducted on young Danish farmers that did not find associations between TLR2, TLR4, or CD14 genotypes and new-onset asthma [25]. Noguchi et al. [26] reported no associations between polymorphisms or haplotype of TLR2 and the risk of asthma. On the other hand, European study showed that farmers' children harboring the T allele in TLR2 –16934 (rs4696480) were less likely to have asthma and current asthma symptoms [14] and another case–control analysis in a Chinese population found that patients with the TLR2 (rs7656411) TT variant homozygote had a significantly reduced risk of asthma when compared with those with the GG wild-type homozygote [27].

Total serum IgE level did not differ with TLR2 genotypes in all studied groups. This was in agreement with a study of 137 asthmatic families in Japan showed that polymorphisms in the TLR2 gene were not associated with IgE [26] and was consistent with a Tunisian study reported that none of the TLRs polymorphisms studied were found to be associated with atopy or total serum IgE level [28].

TLR4 mediates innate immune responses to respiratory syncytial virus which is a common cause of bronchiolitis in infancy

and considered as a risk factor for the atopy and asthma development [29]. Polymorphisms in the coding region of the TLR4 gene at +986A>G position causes amino acid substitutions of Asp299Gly, which affect the extracellular domain of the TLR4 receptor. The TLR4 +986A>G polymorphism results in reduction in cell surface expression of TLR4 and subsequent disruption of LPS mediated signaling [30]. In the current study we could not show any association between the TLR4 polymorphism and the IgE levels or the development of asthma in children. This is in accordance with a heterogeneous American cohort study (Black, White and Hispanic) that found no association between TLR4 polymorphism and the development of asthma and atopy-related traits [13]. Yang et al. [21] reported no association of the TLR4 polymorphism with asthma development. In another study conducted on different polymorphisms of TLR4, they failed to detect any significant association between TLR4 +896A>G and asthma in females [31]. On the other hand, a study carried out on Swedish children found that polymorphism in the TLR4 gene could modify the responsiveness of the host to microbial agents and reported that the TLR4 Asp299Gly polymorphism was associated with a 4-fold higher prevalence of asthma and a 7-fold higher prevalence of atopic asthma [12].

Concerning allergic rhinitis, the genotype and allele frequencies of the TLR2 and TLR4 polymorphisms were not significantly different between allergic rhinitis children and controls. These results are consistent with a case–control study from Korea of 440 patients with allergic rhinitis and 528 controls with no allergic symptoms and negative on allergy testing, the genotype and allele frequencies of the TLR2 and TLR4 polymorphisms were not significantly different between allergic rhinitis children and controls [32]. In contrast, Senthilselvan et al. [33] studied 915 nonsmoking university students from Canada and found that the risk of allergic rhinitis in people carrying minor allele on both SNPs (TLR4 229 and 399) was reduced by 88% in comparison with the TLR4 wild-type group.

Generally, differences between different populations for the risk of TLR2 and TLR4 polymorphisms in allergic diseases may be attributed to the differences in their ethnicity, age, and different environmental exposure. Moreover, the true associations between polymorphisms of a gene and a common disease may not be prominent due to low penetrance of these genes. Multiple gene–gene and gene–environment interactions may be the key in understanding the disease pathogenesis.

To shed a light on the association of TLR2 or TLR4 polymorphisms and disease severity, it was detected that hetero- or homozygosity for the mutant allele of TLR2 or TLR4 polymorphisms were significantly associated with the moderate–severe group compared to the mild group in both atopic asthmatics and allergic rhinitis group whereas no difference was observed in the non-atopic

asthmatics. Interestingly, among all mild asthmatics and mild allergic rhinitis groups, no patient was homozygous for the minor alleles of TLR2 and TLR4 polymorphisms. So, we can consider the polymorphisms of the TLR2 and TLR4 gene may lead to increased atopy severity and this can be explained by both TLR2 +2408A and TLR4 +986G have been associated with hyporesponsiveness to inhaled endotoxin challenge [30] and reduced exposure to microbes enhances the risk of allergic sensitization [34]. Our results are the same of Yang et al. [21] who showed the positive association of TLR4 polymorphism and atopy severity in asthmatics. In contrast, Saçkesen et al. [35] suggested that the presence of TLR4 polymorphisms may confer some protection against severe asthma.

One of the study limitations is that we did not consider the environmental influence through measuring environmental endotoxin levels and so further study is required to provide any data on the gene–environment interaction. In conclusions, polymorphisms in the TLR2 and TLR4 genes may be associated with severe forms of disease in atopic asthmatics and allergic rhinitis patients. But there was no significant association between these polymorphisms and risk of asthma or allergic rhinitis.

Acknowledgments

This work was funded by support of academic research of Zagazig University Projects, Zagazig University Postgraduate & Research Affairs.

References

- [1] D. Price, M. Thomas, Breaking new ground: challenging existing asthma guidelines, *BMC Pulm. Med.* 30 (6) (2006) S1.
- [2] V. Georgy, H.I. Fahim, M. El Gaafary, S. Walters, Prevalence and socioeconomic associations of asthma and allergic rhinitis in northern Africa, *Eur. Respir. J.* 28 (2006) 756–762.
- [3] N. Aberg, J. Sundell, B. Eriksson, B. Hesselmar, B. Aberg, Prevalence of allergic diseases in schoolchildren in relation to family history, upper respiratory infections, and residential characteristics, *Allergy* 51 (4) (1996) 232–237.
- [4] D.P. Strachan, Family size, infection and atopy: the 1st decade of the “hygiene hypothesis”, *Thorax* 55 (Suppl.) (2000) S2–S10.
- [5] S. Akira, K. Takeda, T. Kaisho, Toll-like receptors: critical proteins linking innate and acquired immunity, *Nat. Immunol.* 2 (2001) 675–680.
- [6] L.A. Neill, A.G. Bowie, The family of five: TLR-domain-containing adaptors in Toll-like receptor signaling, *Nat. Rev. Immunol.* 7 (2007) 353–364.
- [7] A.O. Aliprantis, R.B. Yang, M.R. Mark, et al., Cell activation and apoptosis by bacterial lipoproteins through TLR2, *Science* 285 (1999) 736–739.
- [8] A. Ouassii, E. Guilvard, Y. Delneste, G. Caron, G. Magistrelli, N. Herbault, et al., The *Trypanosoma cruzi* Tc52-released protein induces human dendritic cell maturation, signals via Toll-like receptor 2, and confers protection against lethal infection, *J. Immunol.* 168 (2002) 6366–6374.
- [9] S. Sugawara, S. Yang, K. Iki, J. Hatakeyama, R. Tamai, O. Takeuchi, et al., Monocytic cell activation by Nonendotoxigenic glycoprotein from *Prevotella intermedia* ATCC 25611 is mediated by toll-like receptor 2, *Infect. Immun.* 69 (2001) 4951–4957.
- [10] L.K. Williams, D.R. Ownby, M.J. Maliarik, C.C. Johnson, The role of endotoxin and its receptors in allergic disease, *Ann. Allergy Asthma Immunol.* 94 (2005) 323–332.
- [11] M. Fransson, M. Adner, J. Erjefalt, L. Jansson, R. Uddman, L.O. Cardell, Up-regulation of toll-like receptors 2, 3 and 4 in allergic rhinitis, *Respir. Res.* 6 (2005) 100.
- [12] M. Fagerås Böttcher, M. Hmani-Aifa, A. Lindström, M.C. Jenmalm, X.M. Mai, L. Nilsson, H.A. Zdzisek, B. Björkstén, P. Söderkvist, O. Vaarala, A TLR4 polymorphism is associated with asthma and reduced lipopolysaccharide-induced interleukin-12(p70) responses in Swedish children, *J. Allergy Clin. Immunol.* 114 (3) (2004) 561–567.
- [13] B.A. Raby, W.T. Klimecki, C. Laprise, J. Renaud, J. Faith, M. Lemire, C. Greenwood, K.M. Weiland, C. Lange, L.J. Palmer, R. Lazarus, D. Vercelli, D.J. Kwiatkowski, E.K. Silverman, F.D. Martinez, T.J. Hudson, S.T. Weiss, Polymorphisms in toll-like receptor 4 are not associated with asthma or atopy-related phenotypes, *Am. J. Respir. Crit. Care Med.* 166 (2002) 1449–1456.
- [14] W. Eder, W. Klimecki, L. Yu, E. von Mutius, J. Riedler, C. Braun-Fahrlander, D. Nowak, F.D. Martinez, ALEX Study Team, Toll-like receptor 2 as a major gene for asthma in children of European farmers, *J. Allergy Clin. Immunol.* 113 (3) (2004) 482–488.
- [15] P. Ahmad-Nejad, S. Mrabet-Dahbi, K. Breuer, M. Klotz, T. Werfel, U. Herz, K. Heeg, M. Neumaier, H. Renz, The toll-like receptor 2 R753Q polymorphism defines a subgroup of patients with atopic dermatitis having severe phenotype, *J. Allergy Clin. Immunol.* 113 (2003) 565–567.
- [16] M. Werner, R. Topp, K. Wimmer, K. Richter, W. Bischof, M. Wjst, J. Heinrich, TLR4 gene variants modify endotoxin effects on asthma, *J. Allergy Clin. Immunol.* 112 (2003) 323–330.
- [17] American Thoracic Society, Standardization of spirometry, *Am. J. Crit. Care Med.* 152 (3) (1995) 1107–1136.
- [18] Global initiative for asthma, Global strategy for asthma management and prevention, National Institutes of Health, National Heart Lung and Blood Institute, Revised, 2002.
- [19] E.O. Meltzer, Evaluating rhinitis: clinical rhinomanometric and cytological assessments, *J. Allergy Clin. Immunol.* 82 (5) (1988) 900–908.
- [20] S. Ajdary, M.M. Ghamlouie, M.H. Alimohammadian, M. Hosseini, S.R. Pakzad, Lack of association of Toll-like receptor 2 Arg753Gln with cutaneous leishmaniasis, *Parasitol. Int.* 59 (3) (2010) 466–468.
- [21] I.A. Yang, S.J. Barton, S. Rorke, J.A. Cakebread, T.P. Keith, J.B. Clough, S.T. Holgate, J.W. Holloway, Toll-like receptor 4 polymorphism and severity of atopy in asthmatics, *Genes Immun.* 5 (1) (2004) 41–45.
- [22] N. Sudo, S. Sawamura, K. Tanaka, Y. Aiba, C. Kubo, Y. Koga, The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction, *J. Immunol.* 159 (1997) 1739–1745.
- [23] C. Braun-Fahrlander, J. Riedler, U. Herz, W. Eder, M. Waser, L. Grize, et al., Environmental exposure to endotoxin and its relation to asthma in school-age children, *N. Engl. J. Med.* 347 (2002) 869–877.
- [24] R.B. Yang, M.R. Mark, A.L. Gurney, P.J. Godowski, Signaling events induced by lipopolysaccharide-activated toll-like receptor 2, *J. Immunol.* 163 (1999) 639–643.
- [25] L.A. Smit, S.I. Bongers, H.J. Ruven, G.T. Rijkers, I.M. Wouters, D. Heederik, Ø. Omland, T. Sigsgaard, Atopy and new-onset asthma in young Danish farmers and CD14, TLR2, and TLR4 genetic polymorphisms: a nested case-control study, *Clin. Exp. Allergy* 37 (11) (2007) 1602–1608.
- [26] E. Nuguchi, F. Nishimura, H. Fukai, J. Kim, K. Ichikawa, M. Shibasaki, T. Arinami, An association study of asthma and total serum IgE levels for Toll-like receptors polymorphisms in the Japanese population, *Clin. Exp. Allergy* 34 (2004) 177–183.
- [27] F.H. Qian, Q. Zhang, L.F. Zhou, G.F. Jin, J.L. Bai, K.S. Yin, Polymorphisms in the Toll-like receptor 2 subfamily and risk of asthma: a case-control analysis in a Chinese population, *J. Invest. Allergol. Clin. Immunol.* 20 (4) (2010) 340–346.
- [28] J. Lachhab, I.B. Dhifallah, H. Chelbi, K. Hamzaoui, A. Hamzaoui, Toll-like receptors and CD14 genes polymorphisms and susceptibility to asthma in Tunisian children, *Tissue Antigens* 71 (2008) 417–425.
- [29] U. Schauer, S. Hoffman, J. Bittscheidt, A. Köchling, S. Hemmis, S. Bongartz, V. Stephan, RSV bronchiolitis and risk of wheeze and allergic sensitisation in the first year of life, *Eur. Respir. J.* 20 (2002) 1277–1283.
- [30] N.C. Arbour, E. Lorenz, B.C. Schutte, J. Zabner, J.N. Kline, M. Jones, K. Frees, J.L. Watt, Schwartz, TLR4 mutations are associated with endotoxin hyporesponsiveness in humans, *Nat. Genet.* 25 (2000) 187–191.
- [31] K. Adgers, J. Karjalainen, T. Pessi, C. Eklund, M. Hurme, Epistatic effect of TLR4 genes on the risk of asthma in females, *Int. Arch. Allergy Immunol.* 138 (2005) 251–256.
- [32] I. Kang, Y.K. Oh, S.H. Lee, H.M. Jung, S.C. Chae, J.H. Lee, Identification of polymorphisms in the Toll-like receptor gene and the association with allergic rhinitis, *Eur. Arch. Otorhinolaryngol.* 267 (3) (2010) 385–389.
- [33] A. Senthilselvan, D. Rennie, L. Chénard, L.H. Burch, L. Babiuk, D.A. Schwartz, J.A. Dosman, Association of polymorphisms of toll-like receptor 4 with a reduced prevalence of hay fever and atopy, *Ann. Allergy Asthma Immunol.* 100 (5) (2008) 463–468.
- [34] E. Von Mutius, Environmental factors influencing the development and progression of pediatric asthma, *J. Allergy Clin. Immunol.* 109 (2002) s525–s532.
- [35] C. Saçkesen, C. Karaaslan, O. Keskin, N. Tokol, F. Tahan, E. Civelek, O.U. Soyer, G. Adalioglu, A. Tuncer, E. Birben, C. Oner, O. Kalayci, The effect of polymorphisms at the CD14 promoter and the TLR4 gene on asthma phenotypes in Turkish children with asthma, *Allergy* 60 (2005) 1485–1492.