

Effects of Single-Nucleotide Polymorphisms in the TLR7 and TLR9 Genes of Asthmatic Children

Lishen Shan¹, Ping Hou¹, Xinyuan Kang², and Yunxiao Shang¹

¹Shengjing Hospital of China Medical University and ²China Medical University, Shenyang, Liaoning, China

Abstract. Background. To reveal the status of TLR7 and TLR9 SNPs among children and identify correlations between TLR7/TLR9 and asthma susceptibility and phenotypes. **Method.** Genomes were isolated from the blood samples of 100 asthmatic and 104 healthy children. To determine the status of TLR7 (rs179009 and rs5935436) and TLR9 (rs187084 and rs5743836) SNPs, DNA was amplified by PCR and subjected to Sanger sequencing. T and C polymorphisms at nucleotide position -1486 (TLR9 rs187084) was discovered in both groups. **Results.** The frequencies of TT, TC, and CC genotypes at position -1486 were significantly different when the research group was compared to the control group. There were also statistically significant differences in the genotypes at position -1486 based on age and gender following stratification analysis ($p < 0.05$). The rs179009 (T→C) polymorphism of TLR7 was observed in both groups. There was a statistically significant difference in the genotype frequencies at locus rs179009 between the two groups ($p < 0.05$). In addition, there were statistically significant differences in the genotype frequencies at locus rs179009 based on age and gender following stratification analysis ($p < 0.05$). **Conclusion.** Significant correlations between TLR9/TLR7 SNPs and pediatric asthma were identified. The genotypes at TLR7 rs179009 and TLR9 rs187084 were significantly different between the two groups.

Key words: TLR9, TLR7, single-nucleotide polymorphism, mutation, asthma.

Introduction

Asthma is a public health problem of considerable importance that affects over 300 million people worldwide [1] and is likely due to a mixture of genetic and environmental determinants that are poorly understood. Although primarily a disorder resulting from Th2-cell-mediated inflammation, there is significant interest in investigating innate immunity to understand the pathogenesis of this disease [2]. Toll-like receptors (TLRs) are evolutionarily conserved components of the innate immune system that act to directly recognize pathogen-derived elements, including viral and bacterial DNA, lipopolysaccharides, and proteoglycans. They provide a crucial link between the recognition of pathogens by the innate immune system and subsequent activation of adaptive immunity by inducing the maturation of antigen-presenting cells and the differentiation of T cells. TLR9 is expressed in plasmacytoid dendritic cells, B cells, CD3 + CD4 + T cells, and pulmonary epithelial cells [3].

Previous study evaluated the effects of polymorphisms in TLR7 on asthma risk and asthma-related phenotypes in a Chinese Han population (aged 14 to 75 y) with TLR7(rs179009)[4]. It revealed that the genetic variants in TLR7 was associated with asthma-related phenotypes, including eosinophil counts, serum immunoglobulin E levels, lung function, and asthma severity as well. We also choose another TLR7(rs5935436) first analyzed in asthma study (age: 0.8–14). TLR9(rs187084) studied at the age of 6.4 years (mean) in 135 children of Finland. Previous studies reported an association between a single-nucleotide polymorphism (SNP) in the promoter region of TLR9 (rs5743836 or -1237C/T) and asthma in European-American adults [5], Tunisian children [6], and self-reported non-Hispanic white probands [7] and available parents. Contrary to prior reports [5,6], rs5743836 was not associated with asthma in any of the three cohorts [7]. Marginally significant associations were found with FEV1 and FVC ($p = 0.003$ and $p = 0.008$, respectively) in one of the family-based cohorts, but these associations were not significant after correcting for multiple comparisons. Higher promoter activity of the CC genotype was demonstrated by luciferase assay, confirming the functional importance

Address correspondence to Shan Lishen, Shengjing Hospital of China Medical University, 110004, 18940258911, phone: 86 24 96615 52111; e mail: shanls@sj-hospital.org

Table 1. Primer sequences for genotyping *rsl87084*, *rs5743836*, *rsl79009*, and *rs5935436* single nucleotide polymorphisms.

No. Sites	Forward primer	Reverse primer	PCR product length; GC content
S1 <i>rsl79009</i>	TCCCATTTGCTGCCTCTCTT	GCTGCTTCTACCCTCTCGAA	195 bp, GC%:36.92
S2 <i>rs5935436</i>	CCTTCAGTTTCCAACCACGG	TTTCTCTTTGCCCCACCAGA	152 bp, GC%:52.63
S3 <i>rs5743836</i>	GCCTTCACTCAGAAATACCCT	GAGTCAAAGCCACAGTCCACA	553 bp, GC%:52.17
S4 <i>rsl87084</i>	GCCTTCACTCAGAAATACCCT	GAGTCAAAGCCACAGTCCACA	553 bp, GC%:52.17

of this variant. We choose another TLR9 (*rs5743836*) first analyzed in asthma study (age: 0.8–14). The observed associations between *rs5743836* and various immune-mediated diseases in different populations suggest that this SNP may be functional and is potentially involved in immune system regulation.

TLRs can sense a wide range of pathogen-associated molecular patterns from the environment and are capable of mounting immune reactions. They act through complex intracellular signaling cascades. Myeloid differentiation primary response gene-88 (MyD88)-dependent and MyD88-independent mechanisms and AKT pathways contribute to the plasticity of the immune response. In addition, the TLR signaling network is controlled by various regulatory receptors and intracellular signaling modulators. Systematic analyses of TLR genes previously showed that common genetic polymorphisms in TLR7 and TLR9 are associated with asthma [8,9]. TLR7 and TLR9 signaling occurs through the MyD88-dependent pathway [9]. In murine asthma models, TLR9 inhibited ovalbumin-induced inflammation [10]. In addition, the activation of TLR7 prevented the development of inflammation in sensitized mice via the inhibition of both Th1 and Th2 cytokines [10].

A previous study evaluated the effects of the TLR7 *rs179009* polymorphism on the asthma risk and asthma-related phenotypes in a Chinese Han population (ages 14 to 75 years). A second TLR7 polymorphism (*rs5935436*) was investigated in an asthma study (ages 0.8 to 14 years). In addition, the TLR9 *rs187084* polymorphism was studied in 135 children in Finland (mean age 6.4 years). A second TLR9 polymorphism (*rs5743836*) was analyzed in an asthma study (ages: 0.8 to 14 years). Based on these previous studies, we investigated the possible associations of the TLR9 *rsl87084* and *rs5743836* SNPs and TLR7 *rsl79009* and *rs5935436* SNPs with asthma in children.

Materials and Methods

Patients. The study cohort consisted of one hundred children (52 males and 48 females; ages: 0.8 to 14 years) diagnosed at the Shengjing Hospital of China Medical University with asthma based on the guidelines of the Global Initiative for Asthma (GINA) report between February 1, 2010, and October 31, 2011. The control group was composed of 104 healthy volunteers (53 males and 51 females; ages: 0.8 to 14 years). None of the patients or volunteers had a family history of asthma or any other disease, including lung disease (chronic obstructive pulmonary disease or pulmonary fibrosis), allergic inflammation (allergic rhinitis, atopic dermatitis, or allergic purpura), diabetes, nephrotic disease, systemic lupus erythematosus, or psoriasis. Patients with asthma who were treated with rifampicin, phenobarbital, phenytoin, or ephedrine, or those with infection-induced asthma, were not included in this study. Informed consent was obtained for all patients and volunteers. This study was approved by the Medical Ethics Committee of Shengjing Hospital of China Medical University.

DNA isolation. Venous blood samples were collected from all patients and volunteers in EDTA anticoagulant tubes (Thermo Fisher Scientific, Waltham, MA, USA). Genomic DNA was isolated using the TGuide M16 DNA extraction kit (Tiangen Biotech Co., Ltd., Beijing, China) in accordance with the manufacturer instructions, and stored at -20°C until further use.

Genotyping *rsl87084* and *rs5743836* of TLR9, and *rsl79009* and *rs5935436* of TLR7. The genotypes of *rsl87084* and *rs5743836* of TLR9 and *rsl79009* and *rs5935436* of TLR7 were identified by the TaqMan allelic discrimination assay (Thermo Fisher Scientific), which was designed using the Assay-by-Design service (Applied Biosystems, Foster City, CA, USA). The primer sequences, incubation temperatures, and restriction enzymes used to amplify the regions of *rsl87084*, *rs5743836*, *rsl79009*, and *rs5935436*, and the resultant sequence lengths are summarized in **Table 1**. The regions of the *rsl87084*, *rs5743836*, *rsl79009*, and *rs5935436* polymorphisms were amplified using the PCR system detailed in **Table 2**. The PCR mixture was composed of 2 μL /5 ng of genomic DNA, 200 nM probe, 0.9 μM primers, and 2 $\times$ TaqMan

PCR master mix (ABgene; Thermo Fisher Scientific). The DNA fragments were identified by melting curve analysis, and the fragments of interest were genotyped by restriction fragment length polymorphism (RFLP) using the LightScanner High Sensitivity Master Mix (Idaho Technology, Salt Lake City, UT, USA). The RFLP products were separated by electrophoresis on an 8% polyacrylamide gel, which was stained with ethidium bromide for observation under UV light (Pharmacia Biotech ImageMaster VDS; BioSurplus, Inc., San Diego, CA, USA). Known homozygotes and heterozygotes were sequenced and used as internal controls.

Statistical analysis.

SPSS 19.0 (IBM, Armonk, NY, USA) was used to analyze the data.

The genotype and allele frequencies of the SNPs were analyzed using the chi-square test. The conformity of the genotypes and alleles of all SNPs with the Hardy–Weinberg equilibrium (HWE) was confirmed using the online calculator developed by Michael H. Court. Differences with *P*-values <0.05 were considered statistically significant.

Results

Genotype and allele frequencies of the TLR7 and TLR9 SNPs. The genotype and single polymorphic allele frequencies of the rs187084 and rs5743836 SNPs in TLR9 and the rs179009 and rs5935436 SNPs in TLR7 are summarized in **Table 3**. We observed no significant differences in the genotype and allele frequencies of the rs187084 and rs5743836 SNPs of TLR9 or the rs179009 and rs5935436 SNPs of TLR7 between the study subjects and controls. However, the frequencies of the CC and CT genotypes of the rs179009 SNP in TLR7 appeared to be

Table 2. Polymerase chain reaction conditions.

Step	Temperature (°C)	Time	Cycle
Pre-denaturation	95	15 min	
Denaturation	95	15s	40 cycles
Annealing	60	60s	40 cycles
Extension	60	60s	40 cycles
Terminal extension	72	7 min	1 cycle
Storage	4		

Table 3. Frequencies of genotypes and single polymorphic alleles of rs187084 and rs5743836 SNPs of TLR9 and rs179009 and rs5935436 SNPs of TLR7.

			Healthy	Asthma	Total	<i>p</i>
TLR7	rs179009	CC	5	13	18	0.042
		CT	9	14	23	
		TT	90	73	163	
		Total	104	100	204	
	rs5935436	CC	101	94	195	0.314
		CT	3	4	7	
		TT	0	2	2	
		Total	104	100	204	
TLR9	rs5743836	CC	0	0	0	0.46
		CT	0	2	2	
		TT	104	98	202	
		Total	104	100	204	
	rs187084	CC	67	16	83	0
		CT	26	42	68	
		TT	11	42	53	
		Total	104	100	204	

higher in the asthma patients compared to the healthy volunteers. In addition, a greater percentage of asthma patients possessed the CT and TT genotypes of rs187084 in TLR9 compared with the healthy volunteers. The associations between asthma occurrence and the rs179009 or rs187084 SNPs are shown in **Tables 4&5**.

Associations of asthma occurrence with TLR9 rs5935436 after stratification by gender and age.

Females comprised 48% of the study group (48/100 cases). The proportions of females with the TT, CT, and CC genotypes were 47.6% (20/42 cases), 50% (21/42 cases), and 43.75% (7/16 cases), respectively. The results of multivariate Cox model analyses indicated that patients with the CC genotype in TLR9 rs5935436 included a significantly low proportion of females [adjusted hazard ratio (HR) 0.18, 95% confidence interval (CI) 0.06–0.55, *p*=0.003] (**Table 6**). The rates of younger female patients with the TT, CT,

Table 4. Association of asthma occurrence with rs179009 (Chi-square).

SNP	Genotype	Asthma No(n)/Yes(n)	Asthma (%)	Case-total ^a $p < 0.0125^c$	Case-case ^b $P < 0.0125^d$
Total (n=204)		104/100	49.01		
rs179009	TT	89/74	45.39	0.0998	1vs2:0.075
	CT	10/13	56.52	0.0703	2vs3:0.0078
	CC	5/13	72.22	0.0148	1vs3:0.079

^aCase-total: p -values from the comparison between the group of different alleles and the group of 204 total sam-ples;

^bCase-case: p -values from the pairwise comparisons between different genotype groups. Partition chi-square was used to calculated the p -value; ^c P -value was calculated from the " $P = \alpha/2(k-1)$ ", $\alpha = 0.05$, $k = 3$; ^d P value was calculated from the " $P = \alpha/[k(k-1)/2 + 1]$ ", $\alpha = 0.05$, $k = 3$. So the two different P value are 0.0125, if the P values from the comparison between the group of different genotype and the group of total 204 samples or the pairwise comparisons between different genotype group are $< P = 0.0125$, which the statistical analyses were considered significant.

Table 5. Associations of asthma occurrence with rs187084 (Chi-square).

SNP	Genotype	Asthma No(n)/Yes(n)	Asthma (%)	Case-total ^a $p < 0.0125^c$	Case-case ^b $P < 0.0125^d$
Total (n=512)		104/100	49.01		
Rs1050631	TT	11/42	79.25	0.000 ^e	1vs2:0.000
	CT	26/42	61.76	0.017	2vs3:0.0095
	CC	67/16	19.28	< 0.001	1vs3:0.000

^aCase-total: p -values from the comparison between the group of different allele and the group of total 204 samples;

^bCase-case: p -values from the pairwise comparisons between different genotype group. Partition chi-square was used to calculated the p -value. ^c P -value was calculated using " $P = \alpha/2(k-1)$ ", $\alpha = 0.05$, $k = 3$; ^d P -value was calculated using " $P = \alpha/[k(k-1)/2 + 1]$ ", $\alpha = 0.05$, $k = 3$. The two different P -values were 0.0125 if the P values from the comparison between the group of different genotype and the group of total 204 samples or the pairwise comparisons between different genotype group are $< P = 0.0125$, which the statistical analyses were considered significant. ^e P was calculated by Fisher's exact test.

and CC genotypes of TLR9 rs5935436 SNP were 66.7% (18/42 cases), 35.7% (15/42 cases), and 31.25% (5/16 cases), respectively. The results of the multivariate Cox model analyses indicated that patients with the CC genotype in TLR9 rs5935436 included a significantly lower proportion of young female patients compared to the other genotypes (adjusted HR 0.04, 95% CI 0.01–0.15, $p < 0.001$) (Tables 7&8).

Discussion

Asthma is a complex disease involving the cellular secretion of a range of substances, such as cytokines. It is induced by the presence of a high concentration of cortisol in the blood but without the symptoms of Cushing's syndrome. Asthma is a chronic inflammatory disease of the respiratory system, which is determined by a number of factors. These factors include interactions between genetic and environmental factors that result in the development of various complications, such as bronchial

hyperresponsiveness and recurrent episodes of wheezing, coughing, and dyspnea. It is important to determine the level of hypersensitivity of a patient to asthma in clinical practice.

In the present study, we investigated the possible roles of four SNPs (two TLR7 and TLR9 SNPs) in asthma susceptibility and asthma-related phenotypes in a Chinese population. Our results indicate that TLR7/TLR9 SNPs are associated with the risk of asthma. Previous studies found an increased risk of asthma among European Americans with a C allele at position -1237 in the promoter region of TLR9. It was suggested that this effect would be reflected in the distribution of genotypes among patients compared to controls. However, no associations were found between genotype, haplotype, or specific alleles for the TLR9 SNPs at -1237 and 2848 and the risk for myocardial infarction, deep vein thrombosis, or chronic obstructive pulmonary disease among Europeans or asthma among African Americans [5]. In addition, Nancy et al. examined

Table 6. Associations of asthma occurrence with rs187084.

SNP	Genotype	Asthma NO(n)/Yes(n)	Unadjusted		<i>p</i>	Adjusted		<i>p</i>
			HR	95%CI		HR	95%CI	
Total (n=204)		104/100						
rs187084	TT	11/42	1		1			
	CT	26/42	0.42	0.19-0.97	0.423	0.38	0.16-0.90	0.029
	CC	67/16	0.06	0.03-0.15	<0.001	0.05	0.02-0.12	<0.001

The bold values indicate 2-sided *P*-values <0.05 (statistically significant).

^aThe data were calculated by multivariate Cox models and adjusted for gender and age.

Abbreviations: HR, hazard ratio; CI, confidence interval.

Table 7. Associations of Asthma occurrence with TLR9 rs5935436 stratified by gender.

SNP	Genotype	Gender							
		Male				Female			
		Asthma No(n)/ Yes(n)	HR	95%CI	<i>p</i> ^a	Asthma No(n)/ Yes(n)	HR	95%CI	<i>p</i> ^a
Total (n=204)		53/52				51/48			
	TT	1/22	1 (reference)			10/20	1 (reference)		
	CT	5/21	0.15	0.012-1.77	0.99	21/21	0.5	0.19-1.32	0.162
	CC	47/9	0.01	0.001-0.073	0.05	20/7	0.18	0.06-0.55	0.003

The bold values indicate 2-sided *P* value <0.05.

Abbreviations: HR, hazard ratio; CI, confidence interval.

Table 8. Associations of asthma occurrence with TLR9 rs5935436 following age stratification.

SNP	Genotype	Age							
		Elder				Younger			
		Asthma No(n)/ Yes(n)	HR	95%CI	<i>p</i> ^a	Asthma No(n)/ Yes(n)	HR	95%CI	<i>p</i> ^a
Total (n=204)		37/62				67/38			
	TT	4/24	1 (reference)			7/18	1 (reference)		
	CT	13/27	0.35	0.09-1.21	0.096	13/15	0.45	0.14-1.41	0.8
	CC	20/11	0.09	0.025-0.33	<0.001	47/5	0.04	0.01-0.15	<0.001

The bold values indicate 2-sided *P*-values <0.05 (statistical significance).

^aThe data were calculated by multivariate Cox models. Elder (8-14 years), Younger (0-7 years).

Abbreviations: HR, hazard ratio; CI, Confidence Interval.

the association between an SNP in the promoter of TLR9 (rs5743836) and asthma in three unrelated cohorts of asthmatics but found no statistically significant associations after correcting for multiple testing. However, they performed functional studies that appeared to show that this SNP does have functional significance. Despite these in vitro findings, it was suggested that this polymorphism has no significant role in asthma susceptibility or asthma-related phenotypes [7].

A previous study [11] demonstrated that a TLR7 agonist prevented Th2-mediated airway inflammation in an ovalbumin-induced asthmatic murine model, which was consistent with other findings [12–15]. The effects of TLR7 signaling are not limited to animal models of Th2-directed inflammation. For example, it was recently reported that TLR7 function was reduced in adolescents with asthma [16], and there was a deficiency in interferon induced by rhinovirus (a natural ligand of TLR7) in

asthmatic patients but not in well-controlled asthmatics [17,18]. These findings indicate that TLR7 plays a critical role in the pathogenesis of asthma. In addition, Zhang et al. [19] found that the TT genotype of TLR7 rs179010 was overrepresented in female asthmatics compared to controls. However, these associations were not significant after correcting for multiple comparisons. These findings were also inconsistent with the study by Møller-Larsen et al. [20], which suggested that the SNPs in TLR7 are significantly associated with asthma.

The genetic variants of the TLR7 gene that have been identified may alter the function of the encoded protein, and consequently affect lung function and the severity of asthma. The associations between TLR7 polymorphisms in males and females were different. In fact, several investigations have shown sex-specific associations between TLR7 and human diseases [21,22]. However, the exact gender-dependent mechanisms are not yet clear. The difference between the genders may be linked to the X-chromosome location of TLR7, which means that females carry two copies of TLR7 while males only have one copy, and accounts for the consequence of genetic variation and the immunobiology of TLR7 [23]. In addition, the gender-specific differences in hormone secretion, physiological functions, sociocultural attitudes, lifestyle-related factors, and environmental interactions should be taken into consideration [24].

In summary, our study suggests that the polymorphisms in TLR7 and TLR9 are associated with asthma-related phenotypes and asthma risk in the study population. This work increases our understanding of the pathogenesis of asthma in children and could lead to the development of more effective strategies for asthma prevention, prediction, and therapy.

Conclusion. The CC genotype of the rs5743836 SNP of TLR9 was not detected in either asthmatic or control children in Liaoning Province, China. Moreover, significant correlations between TLR9/TLR7 SNPs and pediatric asthma were identified. TLR7 rs179009 and TLR9 rs187084 showed significant differences between asthmatic children and controls.

References

1. Masoli M, Fabian D, Holt S, Beasley R: The global burden of asthma: executive summary of the GINA Dissemination Committee report. *Al-lergy* 2004, 59(5):469-478.
2. Schroder NW, Maurer M: The role of innate immunity in asthma: leads and lessons from mouse models. *Allergy* 2007, 62(6):579-590.
3. Krieg AM: Therapeutic potential of Toll-like receptor 9 activation. *Nat Rev Drug Discov* 2006, 5(6):471-484.
4. Zhang Q, Qian FH, Yin XW, Cao Q, Bai JL, Du Q, Shi Y: Associations of Toll-like receptor 7 and 8 Polymorphisms with Asthma and Asthma-related Phenotypes in a Chinese Han Population. *Iran J Allergy Asthma Immunol.* 2015 Dec;14(6):569-80.
5. Lazarus R, Klimecki WT, Raby BA, Vercelli D, Palmer LJ, Kwiatkowski DJ, Silverman EK, Martinez F, Weiss ST: Single-nucleotide poly-morphisms in the Toll-like receptor 9 gene (TLR9): frequencies, pairwise linkage disequilibrium, and haplotypes in three U.S. ethnic groups and exploratory case-control disease association studies. *Genomics* 2003, 81(1):85-91.
6. Lachheb J, Dhifallah IB, Chelbi H, Hamzaoui K, Hamzaoui A: Toll-like receptors and CD14 genes polymorphisms and susceptibility to asthma in Tunisian children. *Tissue Antigens* 2008, 71(5):417-425.
7. Nancy E Lange, Xiaobo Zhou, Jessica Lasky-Su, Blanca E Himes, Ross Lazarus, Manuel Soto-Quirós, Lydiana Avila, Juan C Celedón, Catherine M Hawrylowicz, Benjamin A Raby, Augusto A Litonjua. Comprehensive genetic assessment of a functional TLR9 promoter polymorphism: no replicable association with asthma or asthma-related phenotypes. *BMC Medical Genetics* 2011, 12:26.
8. Tesse R, Pandey RC, Kabesch M. Genetic variations in toll-like receptor pathway genes influence asthma and atopy. *Allergy* 2011;66:307-16.
9. Pandey RC, Michel S, Tesse R, Binia A, Schedel M, Liang L, Klopp N, Franke A, von Berg A, Bufer A, Rietschel E, Heinzmann A, Laub O, Simma B, Frischer T, Genuneit J, Illig T, Kabesch M. Genetic variation in the Toll-like receptor signaling pathway is associated with childhood asthma. *J Allergy Clin Immunol.* 2013 Feb;131(2): 602-5.
10. Schroder NW, Maurer M. The role of innate immunity in asthma: leads and lessons from mouse models. *Allergy.* 2007. 62:579 – 90.
11. Du Q, Zhou LF, Chen Z, Gu XY, Huang M, Yin KS. Imiquimod, a toll-like receptor 7 ligand, inhibits airway remodeling in a murine model of chronic asthma. *Clin Exp Pharmacol Physiol* 2009; 36(1):43-8.
12. Xirakia C, Koltsida O, Stavropoulos A, Thanassopoulou A, Aidinis V, Sideras P, et al. Toll-like receptor 7-triggered immune response in the lung mediates acute and long-lasting suppression of experimental asthma. *Am J Respir Crit Care Med* 2010; 181(11):1207-16.
13. Van LP, Bardel E, Gregoire S, Vanoirbeek J, Schneider E, Dy M, et al. Treatment with the TLR7 agonist R848 induces regulatory T-cell-mediated suppression of established asthma symptoms. *Eur J Immunol* 2011; 41(7):1992-9.
14. Grela F, Aumeunier A, Bardel E, Van LP, Bourgeois E, Vanoirbeek J, et al. The TLR7 agonist R848 alleviates allergic inflammation by targeting invariant NKT cells to produce IFN-gamma. *J Immunol* 2011; 186(1):284-90.
15. Vultaggio A, Nencini F, Pratesi S, Maggi L, Guarna A, Annunziato F, et al. The TLR7 ligand 9-benzyl-2-butoxy-8-hydroxy adenine inhibits IL-17 response by eliciting IL-10 and IL-10-inducing cytokines. *J Immunol* 2011; 186(8):4707-15.
16. Roponen M, Yerkovich ST, Hollams E, Sly PD, Holt PG, Upham JW. Toll-like receptor 7 function is reduced in adolescents with asthma. *Eur Respir J* 2010; 35(1):64-71.

17. Sykes A, Edwards MR, Macintyre J, del Rosario A, Bakhsoliani E, Trujillo-Torralbo MB, et al. Rhinovirus 16-induced IFN- α and IFN- β are deficient in bronchoalveolar lavage cells in asthmatic patients. *J Allergy Clin Immunol* 2012; 129 (6):1506-1514.e6.
18. Sykes A, Macintyre J, Edwards MR, Del Rosario A, Haas J, Gielen V, et al. Rhinovirus-induced interferon production is not deficient in well controlled asthma. *Thorax* 2014; 69(3):240-6.
19. Qian Zhang, Fen-Hong Qian, Xiao-Wei Yin, Qi Cao, Jian-Ling Bai, Qiang Du, and Yi Shi. Associations of Toll-like Receptor 7 and 8 Poly-morphisms with Asthma and Asthma-related Phenotypes in a Chinese Han Population. *Iran J Allergy Asthma Immunol*. December 2015; 14(6):569-580.
20. Møller-Larsen S, Nyegaard M, Haagerup A, Vestbo J, Kruse TA, Børglum AD. Association analysis identifies TLR7 and TLR8 as novel risk genes in asthma and related disorders. *Thorax* 2008; 63(12):1064-9
21. Shen N, Fu Q, Deng Y, Qian X, Zhao J, Kaufman KM, et al. Sex-specific association of X-linked Toll-like receptor 7 (TLR7) with male systemic lupus erythematosus. *Proc Natl Acad Sci U S A* 2010; 107(36):15838-43.
22. Yue M, Feng L, Tang SD, Wang JJ, Xue XX, Ding WL, et al. Sex-specific association between X-linked Toll-like receptor 7 with the outcomes of hepatitis C virus infection. *Gene* 2014; 548(2):244-50.
23. Schott E, Witt H, Neumann K, Taube S, Oh DY, Schreier E, et al. A Toll-like receptor 7 single nucleotide polymorphism protects from advanced inflammation and fibrosis in male patients with chronic HCV-infection. *J Hepatol* 2007; 47(2):203-11.
24. Osman M, Hansell AL, Simpson CR, Hollowell J, Helms PJ. Gender-specific Page 13 of 19 ESub : 176334 06-10-2016presentations for asthma, allergic rhinitis and eczema in primary care. *Prim Care Respir J* 2007; 16(1):28-35.