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Research paper

Polymorphisms of *TLR7* and *TLR8* associated with risk of asthma and asthma-related phenotypes in a southeastern Chinese Han population ☆

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Abstract

Objective: To evaluate the effects of polymorphisms in *TLR7* and *TLR8*(as potential candidate genes) on asthma risk and asthma-related phenotypes. **Methods:** We consecutively recruited 318 unrelated adult asthmatic patients and 352 healthy volunteers from the same area of southeast China. Genotyping of each selected SNP was performed using multiplex PCR in conjunction with tagged array single base extension technology. We conducted case-control and case-only association studies between the selected SNPs in *TLR7* and *TLR8* and asthma or asthma-related phenotypes. **Results:** The T allele of rs5935436 SNP in *TLR7* was protective from developing asthma in males (adjusted ORs = 0.126, 95% CIs = 0.016-0.995). The CT/TT genotype of rs5935436 was less frequent in female asthmatics with allergic rhinitis (adjusted ORs = 0.18, 95% CIs = 0.04-0.90). The homozygote AA of rs3761623 and GG of rs3764880 were positively associated with lower FEV_1% and asthma severity in female asthmatics. These results were confirmed by haplotype analysis. **Conclusion:***TLR7* and *TLR8* polymorphisms may play an important role in the pathogenesis of asthma that is gender-dependent. This could be clinically useful, both for identifying patients at risk of asthma and for preventing its occurrence.

Key words: Asthma; Atopy; Phenotype; Polymorphism; Toll-like receptors

INTRODUCTION

Asthma is a complex and heterogeneous disorder which is influenced by a number of genetic and environmental factors. Accumulating epidemiological evidence[1,2] supports the hygiene hypothesis[3] that frequent exposure to pathogens or their products in early life can protect against developing asthma or atopy later in life. Although the precise immunological mechanisms of the hygiene hypothesis are still controversial, the concepts that a missing immune deviation of allergen-specific responses from the T helper(Th) type 2 to the Th1 profile and impaired T regulatory cell activity have been proposed[4]. As a bridge linking innate and adaptive immunity, Toll-like receptors(TLRs) skew specific immune responses towards a Th1 or Th2 profile[5] and modulate the function of T regulatory cells

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following binding to diverse conserved motifs in pathogens termed pathogen-associated molecular patterns[6]. Human *TLR7* and *TLR8* genes lie in close proximity to one another on the sex chromosome Xp22[7]. Both of them consist of three exons, and the amino acid residues are encoded by a single major exon, except for the first methionine residue. Their expression products, *TLR7* and *TLR8*, locate exclusively in the endosomal compartment[8], recognize single-stranded RNA, and induce interferons(IFNs) to protect the host from viral infection through a myeloid differentiation factor 88-dependent signaling pathway[9-11]. Our previous studies demonstrated that imiquimod, a synthetic *TLR7*-selective agonist of the imidazoquinoline family, attenuated the airway inflammation and hyperresponsiveness by modulating the Th1/Th2 reaction in an ovalbumin-induced asthmatic murine model[12]. Another imidazoquinoline-like molecule, resiquimod, known as a ligand of *TLR7* and *TLR8*, suppressed acute experimental asthma[13] and chronic airway remodeling[14]. Furthermore, the immunomodulatory activities via *TLR7/8* signaling were confirmed in humans. Resiquimod induced allergen-specific human circulating T cells to produce IFN-γ and even to lose the ability to produce IL-4, thus shifting their cytokine production phenotype to either a Th0 or Th1 profile[15]. These data highlight the fact that *TLR7* and *TLR8* play a critical role in the pathogenesis of asthma.

Studies on the effect of *TLRs* single nucleotide polymorphisms(SNPs) on asthma and asthma-related phenotypes conducted using diverse populations have yielded inconsistent results. Notably, there is still no report about the association between *TLR7* or *TLR8* polymorphisms and asthma. In view of the biological roles of *TLR7* and TLR8 in the development of asthma, we hypothesized that these two genes are potential candidate genes for asthma, and functional genetic variants of them might modify the risk of asthma. To test this hypothesis, we genotyped one polymorphism of *TLR7* and two of *TLR8* in a case-control study in a population of southeastern Chinese Han Nationality.

MATERIALS AND METHODS

Study design

We consecutively recruited 318 adult asthmatic patients from the outpatient department at the First Affiliated Hospital of Nanjing Medical University. Asthma diagnosis was verified and the severity assessed by an experienced pulmonary specialist, according to the Global Initiative for Asthma(GINA) guidelines[16]. All patients had a history of asthma of more than one year and had to satisfy at least one of the following criteria: (1)a diurnal variation in PEF of 20% or more; (2)an increase of =15% or absolute 200 ml in FEV_1 10 to 20 minutes after inhalation of a short-acting β-agonist; (3) a 15% fall in FEV_1 or 20% fall in PEF from baseline 5 to 15 minutes post-exercise. Each patient underwent a physical examination, pulmonary function test, blood test for eosinophil count, chest radiograph, and those examinations necessary to exclude other pulmonary diseases, such as chronic obstructive pulmonary disease(COPD), bronchial carcinoma, lung tuberculosis, cystic fibrosis, a recent upper or lower respiratory tract infection, and foreign bodies. Patients who were pregnant, or had hepatic, renal or cardiovascular disease, diabetes mellitus, cancer status, recent surgery, and systemic inflammatory disorders(such as collagen vascular diseases and inflammatory bowel disease) were also excluded. All patients were categorized as intermittent, mild persistent, moderate persistent, or severe persistent asthma. Atopy was defined by at least one positive response to a skin-prick test(SPT) of 25 common aeroallergens including *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, animal danders, cockroach, pollen, ragweed, mugwort, and moulds(ALK-Abelló A/S, Hørsholm, Denmark). Allergic rhinitis(AR) was defined as rhinitis appearing at least twice after exposure to a particular allergen and not related to infection. A total of 352 healthy volunteers with normal pulmonary function and without personal or family history of asthma and atopy were recruited from spouses of the patients and the general population by advertisements. Each subject completed a questionnaire concerning general information on demographics, the geographic region of origin, medical co-morbidities, smoking, family history, migration status of the participants, clinical history, details of environmental factors, and disease onset. The smokers' cumulative smoking dose(pack-years) was defined as the number of packs of cigarettes smoked per day multiplied by the number of smoking years. Those who had smoked < 5 pack-years were defined as non-smokers, otherwise, they were considered as ever-smokers[17]. All subjects were unrelated Han Chinese residing in Nanjing or the surrounding area, and all signed informed consent for the study procedures. The characteristics of the subjects are detailed in ***Table 1***. The study protocol was approved by the First Hospital Ethics Committee, Nanjing Medical University.

Pulmonary function test

FEV_1 and FVC were measured using a spirometer (MicroLab Spiro V 1.34, Micro Medical Ltd, Rochester Kent, England) by the same technician to ensure the consistency of the maneuver according to the European Respiratory Society standards[18]. The highest value from three technically comparable measurements performed per patient was accepted for subsequent analysis. The

Table 1 **Demographic characteristics of asthmatic patients and control subjects**

	Patients(n=318)	Controls(n=352)	P value
Age (years)*	39.80 ± 14.23	34.27 ± 13.31	< 0.001
Gender n(%)			
Male	135(42.45)	200(56.82)	< 0.001
Female	183(57.55)	152(43.18)	
Smoking n(%)			
Non-smokers	280(88.05)	242(82.88)	0.069
Ever-smokers	38(11.95)	50(17.12)	
Eos($\times 10^6$/ml)*	0.46 ± 0.71	0.14 ± 0.11	< 0.001
$\log_{10}$IgE(IU/ml)*	1.82 ± 0.50	1.19 ± 0.60	< 0.001
FEV_1%(%)*	70.11 ± 25.66	92.75 ± 18.07	0.014
FEV_1%FVC(%)*	68.18 ± 14.78	82.66 ± 9.43	0.006
Atopy n(%)	237(75.00)	0	–
Severity n(%)			
Intermittent	70(22.01)	–	–
Mild persistent	50(15.72)	–	–
Moderate persistent	87(27.36)	–	–
Severe persistent	111(34.91)	–	–
ICS treatment n(%)	175(55.03)	–	–
Family history n(%)			
Yes	120(38.10)	0	–
No	195(61.90)	0	–
Allergic rhinitis n(%)			
Yes	182(57.23)	0	–
No	136(42.77)	0	–
Age at onset (years)*	26.35 ± 15.58	–	–

Eos, eosinophils; $\log_{10}$IgE, $\log_{10}$-transformed immunoglobulin E levels; FEV_1%, percent of predicted forced expiratory volume in 1 second; FEV_1%FVC: FEV_1 as percentage of forced vital capacity; ICS, inhaled corticosteroid. *Data are expressed as mean ± standard deviation(SD). t test or χ^2 test is used as appropriate.

predicted FEV_1 was calculated from regression equations for predicting FEV_1 in normal Chinese subjects.

Measurement of total serum IgE

The levels of total serum IgE were measured using a human IgE ELISA quantitation kit and a starter accessory package(Bethyl Laboratories, Montgomery, TX, USA) according to the manufacturer's instructions. The minimum detection level was 6.51 IU/ml.

SNPs selection

We searched the *TLR7* and *TLR8* SNPs in the International Hapmap Project(http://www.hapmap.org). Based on the Tagger-pairwise Tagging algorithm, 7 tagging SNPs in *TLR7* and 6 tagging SNPs in *TLR8* were picked out from the Chinese Han population in Beijing (CHB) with the cutoff of $r^2 = 0.8$ and minor allele frequency(MAF) above 0.05. As a preliminary study, we selected and genotyped 3 SNPs from the tagging SNPs, namely rs5935436(−1311C/T), rs3761623(−2650A/G) and rs3764880(+69G/A). The rs5935436 polymorphism is located in the promoter of *TLR7*, while the rs3764880 is located in the 5' untranslating region of *TLR8* exon 1, and 2724 bases upstream from the rs3764880 is rs3761623.

Genotyping

Genomic DNA was extracted from ethylenediamine tetraacetic acid(EDTA)-anticoagulated peripheral blood by using the QIAamp DNA Blood Mini kit(Qiagen, Valencia,CA,USA) according to the manufacturer's instructions. We genotyped these 3 SNPs by using multiplex PCR in conjunction with tagged array single base extension genotyping technology with the GenomeLab SNPstream genotyping platform(Beckman Coulter, Fullerton, CA, USA) and the SNPstream software as described previously[19]. The PCR primers and extension probes with the tag sequence were designed using the web-based Autoprimer design tool (http://www.autoprimer.com), and were synthesized by SBSgene (SBS Genetech Technology, Shanghai, China) (see details in ***Table 2***). We duplicated 10% samples to confirm the concordance and accuracy of genotyping. A sample call rate > 99% was observed with 100% matching in the replicates.

Statistical analysis

The differences in the demographic and clinical data between patients and controls were compared by using t tests or χ^2 tests as appropriate. Because either *TLR7* or *TLR8* was located on the X-chromosome, and males therefore have only one copy, Hardy-Weinberg equilibrium was tested for genotype frequencies in female cases and female controls respectively using a goodness-of-fit χ^2 test. All analyses of the X-chromosome-

Table 2 **PCR primers and extension probes**

SNPs*	Primer	Sequence 5'-3'
rs5935436	Forward	AAACCCAACCAGAAGCCA
(−1311C/T)	Reverse	AAGCTTTCTCTTTGCCCC
	Probe	AGGGTCTCTACGCTGACGATGGCTACTGATGAAGCACATATAGCT
rs3761623	Forward	ATTGTCTGGTCATCGACTCG
(−2650A/G)	Reverse	TAGCAGAGCTGGAATTATTATTACTACG
	Probe	CGACTGTAGGTGCGTAACTCTTGGCAACAGCCCAGGAATGTGTGA
rs3764880	Forward	TACCATTCTGCGCTGCTG
(+69G/A)	Reverse	ACACCATTTGCCACCAAC
	Probe	AGATAGAGTCGATGCCAGCTAATGAAAAATTAGAACAACAGAAAC

*Nucleotide numbering starts from the transcriptional initiation point. SNPs, single nucleotide polymorphisms.

located *TLR7* or *TLR8* were conducted in males and females separately. Logistic regression was used to estimate crude and adjusted odds ratios(ORs) and 95% confidence intervals(CIs) as a measure of association with the risk of asthma. The *t* test, χ^2 test, linear regression and logistic regression were applied in the case-only study. EM algorithm in SAS 9.1.3 PROC HAPLOTYPE was used to infer haplotype frequencies based on the observed genotypes. A significance level was defined as 0.05 and all calculations were carried out with SAS 9.1.3(SAS Institute, Cary, NC, USA).

RESULTS

The genotype distribution was in Hardy-Weinberg equilibrium for all SNPs in the female patient and control groups($P > 0.05$), except for rs3761623 in the female controls($P = 0.028$). Genotype and allele frequencies of each SNP are shown in ***Table 3***. The T allele of the rs5935436 was more frequent in male control subjects than in male patients(crude $P = 0.034$). Even after adjustment for age and smoking status, it was still statistically significant(adjusted $P = 0.049$). Logistic regression revealed that male individuals who carried T allele had a significant 87.4% reduced risk of asthma compared with those with C allele(95% CIs = 0.016-0.995). This change was still significant when the comparison was performed just in non-smokers. The protective T allele was also more frequent in female controls than in female asthmatics. However, the difference was not significant between the two groups after correction(adjusted ORs = 0.432, 95% CIs = 0.183-1.017, $P = 0.055$). There seemed to be similar allele frequencies of rs3761623 and rs3764880 among patients and controls in both males and females($P > 0.05$)(***Table 3***). Taking the confounding factor of atopy into consideration, we stratified asthmatic subjects into two groups, designated as atopic asthma and nonatopic asthma. No difference of allele or genotype distribution for each SNP was observed between cases and controls either in males or in females. Also, when asthmatic subjects were stratified using the sorting criterion of family history of asthma, no positive results were found(data not shown). However, male late-onset asthmatics(age at asthma onset > 13 years), but not early-onset asthmatics(age at asthma onset = 13 years), had lower frequencies of the rs5935436 T allele than male controls(adjusted ORs = 0.14, 95% CIs = 0.00-0.87, $P = 0.032$). Furthermore, we divided asthmatic subjects into two subgroups on the basis of patient history of allergic rhinitis. The data showed that the G allele of the rs3761623 was more frequent in male asthmatics without allergic rhinitis(adjusted ORs=3.78,95% CIs = 1.02-14.04, $P = 0.047$), and the CT/TT genotype of the rs5935436 was less frequent in female asthmatics with allergic rhinitis(adjusted ORs = 0.18, 95% CIs = 0.04-0.90, $P = 0.037$).

Next, we analyzed the distribution of haplotypes and their effects on asthma. Haplotypes were reconstructed incorporating all three SNPs, namely, rs5935436, rs3761623, and rs3764880 in order. A total of five haplotypes were generated in female subjects and only four were deduced in males(***Table 4***). Female but not male carriers of the most frequent CAG haplotype had a 1.52-fold(95%CIs=1.044-2.22) increased risk of developing asthma than those of non-CAG, with a *P* value of 0.029 adjusted for age and smoking status. On the other hand, the TAG haplotype was less frequent in both female and male subjects with a protective effect on asthma, though the difference was marginally significant in males($P = 0.049$) and was not statistically significant in females after adjustment($P = 0.055$) (***Table 4***).

Subsequently, we focused on the associations of genotypes and haplotypes with asthma-related phenotypes in a case-only study. As shown in ***Table 5***, female patients with AA homozygote of rs3761623 had significantly lower FEV_1% than those with AG/GG genotype(67.89 ± 25.25 vs. 76.92 ± 23.75, $P = 0.031$ adjusted for age, smoking status, inhaled corticosteroid treatment, and atopy). Besides, the overrepresentation of AA genotype in moderate/severe asthmatic patients revealed a positive association of AA genotype with asthma severity. Such associations were more evident

Table 3 **Allele and genotype frequencies of *TLR7* and *TLR8* polymorphisms in patients and controls and risks of asthma**

SNPs	Allele/Genotype	Patients(%)	Controls(%)	ORs(95%CIs)*	*P* value*
Male		*n*=135	*n*=200		
rs5935436	C	134(99.26)	189(94.50)	1.000	
	T	1(0.74)	11(5.50)	0.126(0.016–0.995)	0.049
rs3761623	A	111(83.46)	173(88.27)	1.000	
	G	22(16.54)	23(11.73)	1.395(0.736–2.641)	0.307
rs3764880	G	110(82.71)	168(85.28)	1.000	
	A	23(17.29)	29(14.72)	1.165(0.637–2.132)	0.619
Female		*n*=183	*n*=152		
rs5935436	C	357(97.54)	287(94.41)	1.000	
	T	9(2.46)	17(5.59)	0.432(0.183–1.017)	0.055
	CC	174(95.08)	136(89.47)	1.000	
	CT	9(4.92)	15(9.87)	0.453(0.186–1.104)	0.081
	TT	0(0.00)	1(0.66)	–	–
	CT/TT	9(4.92)	16(10.53)	0.435(0.180–1.051)	0.064
rs3761623	A	312(85.25)	243(80.46)	1.000	
	G	54(14.75)	59(19.54)	0.756 (0.497–1.151)	0.192
	AA	134(73.22)	102(67.55)	1.000	
	AG	44(24.04)	39(25.83)	0.857(0.510–1.439)	0.560
	GG	5(2.73)	10(6.62)	0.469(0.148–1.489)	0.199
	AG/GG	49(26.78)	49(32.45)	0.787(0.482–1.283)	0.336
rs3764880	G	307(84.34)	240(78.95)	1.000	
	A	57(15.66)	64(21.05)	0.746(0.496–1.124)	0.162
	GG	129(70.88)	98(64.47)	1.000	
	GA	49(26.92)	44 (28.95)	0.850(0.514–1.404)	0.525
	AA	4(2.20)	10(6.58)	0.410(0.122–1.384)	0.151
	GA/AA	53(29.12)	54(35.53)	0.777(0.482–1.254)	0.302

SNPs, single nucleotide polymorphisms; ORs, odds ratios; CIs, confidence intervals. *Adjusted for age and smoking status. Logistic regression is used.

Table 4 **Haplotype frequencies of *TLR7* and *TLR8* polymorphisms in patients and controls and risks of asthma**

Haplotype	Patients(%)	Controls(%)	ORs (95%CIs)*	*P* value*
Male				
CAA	1 (0.75)	6(3.06)	0.278(0.033–2.369)	0.242
CAG	109 (81.95)	156(79.59)	1.207(0.684–2.131)	0.515
CGA	22 (16.54)	23(11.73)	1.395(0.736–2.641)	0.307
TAG	1 (0.75)	11(5.61)	0.125(0.016–0.990)	0.049
Female				
CAA	6 (1.65)	6(2.02)	0.881(0.265–2.926)	0.836
CAG	297 (81.13)	221(72.69)	1.523(1.044–2.222)	0.029
CGA	52 (14.19)	58(19.03)	0.752(0.492–1.150)	0.188
TAG	9 (2.46)	17(5.59)	0.432(0.183–1.017)	0.055
CGG	2 (0.57)	2(0.67)	0.732(0.085–6.338)	0.777

ORs, odds ratios; CIs, confidence intervals. *Adjusted for age and smoking status. Logistic regression is used. Haplotypes formed by rs5935436, rs3761623, and rs3764880 in order.

for the GG genotype of rs3764880 polymorphism when compared with GA/AA genotype. Moreover, the CAG haplotype was positively associated with lower FEV_1% (adjusted $P = 0.031$). However, no associations were found between these two SNPs and other asthma-related phenotypes, such as eosinophil counts and total serum IgE levels. In addition, no association existed between haplotype or genotype of each SNP and asthma-related phenotypes in male asthmatics(data not shown).

DISCUSSION

TLR7 and *TLR8* play an important role in innate and adaptive immunity and mediate anti-viral immunity[9,20]. Ligation of *TLR7* or *TLR8* results in the activation of nuclear factor-κB and the secretion of proinflammatory cytokines such as IFN-α tumor necrosis factor-α and IL-12[2,11], shifting the specific immune responses toward Th0 or even Th1 responses[15,21]. Also, *TLR7* or *TLR8* signaling exerts direct roles in the activation of T regulatory cells, eosinophils, and B cells[6,22,23]. Accumulating evidence shows that synthetic

Table 5 **Association of genotypes and haplotypes of *TLR7* and *TLR8* polymorphisms with asthma-related phenotypes in female asthmatics**

	Eos($\times 10^6$/ml) (mean ± SD)	FEV_1%(%) (mean ± SD)	log_{10}IgE (IU/ml) (mean ± SD)	Asthma severity	
				Intermittent/Mild	Moderate/Severe
rs5935436					
CC	0.47 ± 0.78	70.18 ± 25.40	1.80 ± 0.50	66	108
CT/TT	0.29 ± 0.23	72.22 ± 20.05	1.71 ± 0.54	4	5
P_1	0.085*	0.813*	0.641*	0.734‡	
P_2	0.433†	0.991†	0.486†	0.980§	
rs3761623					
AA	0.46 ± 0.86	67.89 ± 25.25	1.77 ± 0.50	46	88
AG/GG	0.44 ± 0.33	76.92 ± 23.75	1.84 ± 0.48	24	25
P_1	0.835*	0.032*	0.437*	0.071‡	
P_2	0.839†	0.031†	0.700†	0.045§	
rs3764880					
GG	0.44 ± 0.82	67.14 ± 25.17	1.76 ± 0.50	44	85
GA/AA	0.51 ± 0.58	78.75 ± 22.85	1.86 ± 0.48	26	27
P_1	0.490*	0.005*	0.272*	0.060‡	
P_2	0.581†	0.003†	0.499†	0.029§	
Haplotype					
CAG	0.45 ± 0.80	68.84 ± 25.29	1.78 ± 0.50	107	190
Non-CAG	0.49 ± 0.52	76.53 ± 23.35	1.84 ± 0.47	33	36
P_1	0.675*	0.023*	0.380*	0.069‡	
P_2	0.765†	0.031†	0.835†	0.090§	

*Eos, eosinophils; log_{10}*IgE, log_{10}-transformed immunoglobulin E levels; FEV_1%, percent of predicted forced expiratory volume in 1 second. P1, P values determined using *t* tests* and χ^2 tests ‡.P2, P values determined using linear regression adjusted for age, smoking status, inhaled corticosteroid treatment, and atopy †, and logistic regression adjusted for age, smoking status, inhaled corticosteroid treatment, and atopy §. Haplotypes were formed by rs5935436, rs3761623, and rs3764880 in order.

TLR7/8 agonists can effectively suppress acute and chronic experimental asthma[12,13,14].

Genetic variants in *TLR* genes have been extensively investigated for association with asthma or atopy. Most of the studies focused on *TLR4,* showing incompatible results in different populations. Fageras Bottcher and colleagues[24] reported a common variant, A896G, in *TLR4* associated with a 4-fold higher risk of asthma development in Swedish children, and this was confirmed in a study of Turkish children conducted by Sackesen *et al*[25] showing a higher frequency of 896G allele in the mild asthma group. However, no association was reported in a UK Caucasian population between A896G polymorphism and asthma[26]. In addition to *TLR4*, associations of polymorphisms of *TLR2*[27,28], *TLR3*[27], *TLR6*[29], *TLR9*[27,30] and *TLR10*[31] with asthma were also investigated in diverse populations. However, no study has focused on associations between *TLR7* or *TLR8* polymorphisms and asthma development. There is only one study[32] demonstrating a protective role for the *TLR7* G allele of c.1-120T>G in the development of inflammation and fibrosis in male patients with chronic hepatitis C virus infection, of which the immunopathogenesis was related to Th1/Th2 responses.

In the present study, we found that the rs5935436 T allele(−1311T) of *TLR7* was protective in the development of asthma in males. However, when we stratified asthmatic subjects by age at asthma onset[33], this protective role was only seen in male late-onset asthma (age at asthma onset > 13 years), but not in early-onset asthma(age at asthma onset = 13 years). No difference in the T allele distributions was detected between cases and controls when patients were stratified by atopy or family history of asthma. Interestingly, the CT/TT genotype of the rs5935436 reduced the risk 82% of developing allergic rhinitis in female asthmatics, suggesting a protective effect mediated by T allele in allergic rhinitis. Although the reasons for these changes were not clear and there was little functional evidence about rs5935436 polymorphism, it is biologically plausible that the base transition(C > T) at this locus might modulate the function of the *TLR7* promoter, which would influence the *TLR7* gene expression. Further functional studies on the rs5935436 polymorphism are obviously warranted.

Based on the similarities between *TLR7* and *TLR8* in phylogenetics, structure and location[7], we reconstructed the haplotypes consisting of the selected SNPs in both genes. As a consequence, the most frequent CAG haplotype exerted a 1.52-fold increased risk of developing asthma in females compared with other haplotypes. The TAG haplotype, on the other hand, showed a weak protection from asthma in males, and we presumed this effect was attributed to the rs5935436 T allele. Although the case-control study did not

disclose any association between the other two SNPs, rs3761623 and rs3764880, in *TLR8* and asthma, the case-only study underscored a positive association between the homozygous AA genotype of rs3761623 or GG genotype of rs3764880 and the lower FEV_1%, or more severe form of asthma in female, but not male, asthmatic patients. No association was found between each SNP and eosinophil counts or total serum IgE levels.

Interestingly, we observed that *TLR7* and *TLR8* SNPs differentially influenced asthma and asthma-related phenotype dependence on gender. This gender-based difference may be linked to the X-chromosome location of *TLR7/8*. It is clear that the females carry two copies of *TLR7/8* whereas only one copy is present in males, accounting for the consequence of genetic variation and the immunobiology of *TLR7/8*[32]. Indeed, the variant alleles were rarely identified as a homozygote in female asthmatics in the current study, suggesting a more prominent recessive trait in men than in women. Moreover, most genes on one X-chromosome are silenced due to the X-chromosome inactivation and approximately 15% of X-linked genes escape inactivation to some degree[34], affecting the penetrance of a mutation in female mammals. Nevertheless, the gender-specific differences in hormone secretions, physiological functions, socio-cultural attitudes, and lifestyle exposures to the environment should also be taken into considerations[35]. Actually, some elegant studies have demonstrated the associations between asthma and other X-linked genes polymorphisms that are dependent on gender, such as cysteinyl-leukotriene type-1 receptor[36], chemokine(C-X-C motif) receptor 3[37] and tissue inhibitor of metalloproteinase-1[38].

To our best knowledge, this is the first study to investigate the association between *TLR7* and *TLR8* polymorphisms and asthma as well as asthma-related phenotypes. It should be noted that the sample size was relatively small for some genotype distributions after stratification by gender, which may have biased our results. Only three SNPs were selected in this preliminary investigation, and thus, other potential functional polymorphisms in coding regions which may influence the activity of *TLR7* and *TLR8* were not considered. Moreover, functional studies on *TLR7* and *TLR8* polymorphisms were not performed. Therefore, further functional studies, and larger well-designed prospective studies are warranted to explore the exact biological mechanisms of the effects of *TLR7* and *TLR8* genotypes and haplotypes on asthma development in diverse populations.

In summary, we present the first investigation of the association between *TLR7* and *TLR8* polymorphisms and asthma and asthma-related phenotypes. Our findings suggest that *TLR7* and *TLR8* polymorphisms may play an important role in the pathogenesis of asthma gender-dependence. Clinically, this information might be applicable to identifying patients at risk of asthma, and could be used in the prevention of its development.

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