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# Associations of Toll-like Receptor 7 and 8 Polymorphisms with Asthma and Asthma-related Phenotypes in a Chinese Han Population

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## ABSTRACT

Toll-like receptor (TLR) 7 and 8 mediate anti-virus immunity and are of particular relevance to asthma. However, very little information about genetic association on TLR7/8 and asthma are available.

This study aimed to evaluate the effects of polymorphisms in TLR7 and 8 on asthma risk and asthma-related phenotypes in a Chinese Han population. We enrolled 462 unrelated adult asthmatic patients and 398 healthy volunteers. The genotypes of tagging single nucleotide polymorphisms (SNPs) in TLR7 and 8 genes were determined using multiplex SNaPshot SNP genotyping assay.

We used case-control and case-only studies to assess any links with asthma and asthma-related phenotypes. There was no association between the variants in TLR7 and *8* and asthma susceptibility. However, our results revealed that the genetic variants in TLR7 and 8 were associated with asthma-related phenotypes, including eosinophil counts, serum immunoglobulin E levels, lung function, and asthma severity as well. Our study suggests that TLR7 and *8* polymorphisms may play a considerable role in the pathogenesis of asthma.

It will help in better understanding the pathogenesis of asthma and development of more effective strategies for asthma prevention, prediction, and therapy.

**Keywords:** Asthma; Allergy; Genotype; Polymorphism; Toll-like receptor 7 and 8

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## INTRODUCTION

Asthma is an inflammatory disorder of the airways frequently characterized by excessive T helper (Th) type 2-biased immune responses.[1] Although the precise mechanisms remain poorly understood, it is widely believed that asthma is the result of a complex interaction between environmental factors and genetic variants.[2]

Epidemiologic data suggest that the imbalance between Th2 and Th1 immune responses may be the result of reduced childhood microbial exposure.[3] Microbes are detected by a set of specific pattern-recognition receptors of the innate immune system, termed Toll-like receptors (TLRs). TLRs are activated by highly conserved molecular motifs on invading microorganisms and play an essential role in activation of the innate and adaptive immune system.[4] TLRs signal through nuclear factor-κB (NFκB), mitogen-activated protein (MAP) kinases and interferon regulatory factors 3 (IRF-3), to produce many inflammatory cytokines and type I interferon.[5] In turn, they activate antigen-presenting cells (APCs), influence T cell polarization and development,[6] and modulate the function of regulatory T cells,[7] all of which are key events involved in the pathogenesis of asthma. A lack of TLRs activation and Th1 responses during critical periods of immune system maturation may allow Th2 adaptive immunity to predominate.

TLR7 and TLR8, two highly homologous and phylogenetically similar members of TLRs family, are of particular relevance to asthma. They are expressed in bronchial epithelial cells,[8] airway smooth muscle cells[9] and several immune cell types. TLR7 and TLR8 are located intracellularly in the endosome and bind to single-stranded viral RNA, a molecular motif common to many respiratory viruses.[10] Activating TLR7 and TLR8 signaling pathway results in a shift of the immune response toward Th1 and clearance of virus. Accumulating evidence demonstrates that TLR7 and TLR8 stimulations possess the potential of anti-asthma and asthma related allergic diseases, not only in animal models but also in asthmatic patients.[11-15] Furthermore, a recent study revealed that stimulating TLR7 and TLR8 with agonists could result in a rapid relaxation of methacholine-contracted human airways in vitro.[16]

Human TLR7 and TLR8 genes are located in close proximity to one another on the sex chromosome Xp22.[17] 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. Several studies reported the relationship between single nucleotide polymorphisms (SNPs) in TLR7/8 and human disease, such as systemic lupus erythematosus, hepatitis C virus infection, Behçet's disease and HIV-1 disease.[18-21] However, rare data on asthma and asthma related allergic diseases are available. Nilsson et al.[22] reported that the genetic variations in the TLR7/8 gene regions influenced the risk and degree of allergic rhinitis in one Swedish and one Chinese population. In addition, a research in Denmark indicated that the SNPs in TLR7/8 conferred susceptibility to asthma and asthma related atopic disorders.[23] Consistently, our previous study[24] selected one SNP in TLR7 and two in TLR8 in a Chinese population and found that TLR7/8 polymorphisms contributed to asthma susceptibility. Thus, the aim of this study was to investigate the association between haplotype tagging SNPs (capturing all essential genetic information of the TLR7 and TLR8 genes) and asthma as well as asthma-related phenotypes in another independent sample of Chinese Han population.

## MATERIALS AND METHODS

### Subjects

We enrolled 462 asthmatic patients (aged 14 to 75 y) and 398 non-asthmatic controls (aged 16 to 74 y) from January 2011 to May 2013 (Table 1). All subjects were unrelated Han Chinese residing in Changzhou and the surrounding regions. The asthmatics were consecutively recruited from the outpatient department at the Changzhou No.2 People's Hospital affiliated to Nanjing Medical University. Asthma diagnosis and severity were verified by an experienced pulmonary specialist according to the Global Initiative for Asthma (GINA) guidelines.[25] The patients were categorized into 4 groups based on their clinical features (stage 1, intermittent; stage 2, mild-persistent; stage 3, moderate-persistent; and stage 4, severe-persistent). Each patient underwent a detailed workup, including medical history, family history, smoking habit, occupation, general physical examination, medication, skin prick test (SPT), complete blood count, and extended laboratory tests. The healthy volunteers were recruited from the general population without family history of

asthma, and had to meet the following criteria: (a) good health status and matched with the cases for age and area of residence; (b) no positive result for SPT; and (c) normal levels of total serum immunoglobulin (Ig) E. All subjects signed informed consent for the study procedures. The study protocol was approved by the institutional ethics (No. 201089) committee.

**Assessment of Clinical Data**

Atopy was defined as at least one positive response to a SPT of 13 common aeroallergens, including *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Felis domesticus*, *Canis familiaris*, cockroach, pollen, ragweed, mugwort, moulds (*Cladosporium* and *Alternaria*) and animal allergens (cat, dog, and horse). Participants who had smoked at least 100 cigarettes in their lifetimes were defined as smokers; otherwise, they were considered as nonsmokers.[26] SPT, total serum IgE levels, and pulmonary function assessments were described in detail in our previous study.[24,27]

**SNPs Selection and Genotyping**

Haplotype tagging SNPs were selected from the HapMap database (http://www.hapmap.org) using the algorithm-Tagger pairwise Tagging (HapMap Data Rel 27 Phase II+III, Feb 09 2011, on NCBI B36 assembly, dbSNP b126). Based on the largest number of SNPs with a minor allele frequency (MAF) >0.05 in the Chinese Han population (CHB) in Beijing and linkage disequilibrium ($r^2$ >0.8), eight haplotype tagging SNPs in TLR7 and six in TLR8 capturing the genetic information of all common SNPs at the locus were selected for genotyping (Table 2). Genomic DNA was extracted from EDTA-anticoagulated peripheral blood with the QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. We genotyped the SNPs by multiplex SNaPshot technology using an ABI fluorescence-based assay discrimination method (Applied Biosystems, Foster city, CA, USA), which had been described in detail in previous studies [28, 29] at Shanghai Geneskies Biotech co., LTD (Shanghai, China). The PCR primers and extension probes with the tag sequence were designed using the web-based Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Detection of single base extended probe primers was based on fluorescence and extended length detected by capillary electrophoresis on ABI3730XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The data were analyzed with GeneMapper analysis software version 4.1 (Applied Biosystems, Foster City, CA, USA). We duplicated 10% samples to confirm the concordance and accuracy of genotyping. A sample call rate >99% was observed with 100% matching for quality control samples and blind replicates.

**Statistical Analysis**

Differences in the distribution of demographic characteristics, clinical data and genotypes of the TLR7/TLR8 variants between controls and cases were evaluated using the *t* test, $\chi^2$ test and the Fisher exact test as appropriate. The Hardy-Weinberg equilibrium (HWE) was tested using the $\chi^2$ goodness-of-fit test to compare the observed genotype frequencies with the expected frequencies in female controls. Logistic regression was used to estimate crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs), adjusted for age and smoking status, as a measure of association with the risk of asthma. Linear regression and logistic regression, adjusted for age, smoking status, inhaled corticosteroid treatment, and atopy, were applied in the case-only study. Three genetic models were used for statistical analysis, including dominant model, recessive model and additive model. As either TLR7 or TLR8 is located on X-chromosome, and males therefore have only one copy, all analyses were conducted in males and females separately. We estimated the false-discovery rate (FDR) for multiple comparisons, and FDR-corrected *P* values ($P_{FDR}$) of less than 0.05 were considered to be significant. Statistical analysis was carried out with SAS 9.1.3 (SAS Institute, Cary, NC, USA).

## RESULTS

**Demographic and Clinical Characteristics**

The demographic and clinical characteristics of the study population were summarized in Table 1. No significant differences were observed between the controls and cases for age and smoking habit. However, differences in peripheral eosinophil counts, serum IgE, percent of predicted forced expiratory volume in 1 second ($FEV_1$%) and $FEV_1$ as percentage of forced vital capacity ($FEV_1$/FVC) were pronounced between the controls and the cases (all $p$<0.001).

**TLR7 and 8 Gene Polymorphisms Genotype Distributions**

The distribution of each genetic variant met the conditions of the HWE (Table 2). MAF of the fourteen SNPs from the present study, as well as from the HapMap CHB populations, were shown in Table 2. Regrettably, there seemed to be similar allele distributions of each SNP between controls and cases in males ($p$>0.05). We compared the genotype distributions of each SNP between controls and cases in females using dominant, recessive and additive genetic models, respectively. A significant difference was observed in the distributions of rs179010 between controls and cases when a recessive genetic model was used. The TT genotype of rs179010 was more frequent in female asthmatics, when compared with controls (OR=2.18, 95% CI=1.14-4.14, $p$=0.018). But these associations were not significant after correcting for multiple comparisons ($P_{FDR}$=0.180). And no associations were found between other SNPs and risk of asthma.

**Genotypes of the TLR7 and 8 and Asthma-related Phenotypes**

Subsequently, we put the asthma-related phenotypes, such as eosinophil counts, total serum IgE levels, $FEV_1$%, $FEV_1$/FVC and asthma severity, into a case-only analysis. As shown in Table 3, in male asthmatics, significant associations were seen between 6 SNPs (rs179009, rs179010, rs1634322, rs2159377, rs17256081, and rs4830805) and either increased or decreased eosinophil counts. Likewise, 3 SNPs (rs1620233, rs179012, and rs5741883) were associated with total serum IgE levels. Moreover, male asthmatics, who carried the minor allele of rs179012, had significantly higher $FEV_1$% and $FEV_1$/FVC, whereas, those who carried the minor allele of rs2159377, had poorer lung function.

**Table 1. Demographic characteristics of subjects**

| | Controls ($n$ = 398) | Patients ($n$ = 462) | $p$ value |
|---|---|---|---|
| Age (years)* | 43.86±13.31 | 44.74±15.04 | NS |
| Gender $n$ (%) | | | |
| Male | 231(58.04) | 168(36.36) | <0.001 |
| Female | 167(41.96) | 294(63.64) | |
| Smoking $n$ (%) | | | |
| Non-smoker | 322(80.90) | 384 (83.12) | NS |
| Smoker | 76 (19.10) | 78(16.88) | |
| Eos ($\times 10^6$/ml)* | 0.14±0.11 | 0.35±0.89 | <0.001 |
| IgE (IU/ml)* | 37.54±57.70 | 136.75±139.45 | <0.001 |
| $FEV_1$% (%)* | 92.25±19.33 | 70.90±22.37 | <0.001 |
| $FEV_1$/FVC (%)* | 83.16±12.42 | 67.49±16.40 | <0.001 |
| Atopy $n$ (%) | 0 | 268 (58.10) | – |
| Severity $n$ (%) | | | |
| Intermittent | – | 134(29.39) | – |
| Mild persistent | – | 144(31.58) | – |
| Moderate persistent | – | 76(16.67) | – |
| Severe persistent | – | 102(22.37) | – |
| ICS treatment $n$ (%) | – | 196(42.40) | – |

Abbreviations: Eos, eosinophil; $FEV_1$%, percent of predicted forced expiratory volume in 1 second; $FEV_1$/FVC: $FEV_1$ as percentage of forced vital capacity; ICS, inhaled corticosteroid; IgE, immunoglobulin E levels; NS, non-significant.

*Data are expressed as mean ± standard deviation (SD). $t$ test or $\chi^2$ test is used appropriately.

**Table 2. Tagging SNPs selected from HapMap**

| Tagging SNPs (NCBI rs Number) | Base Change | HWE | Genenotyped Call Rate (%) | MAF | | |
|---|---|---|---|---|---|---|
| | | | | Present (Controls) | Present (Patients) | HapMap-CHB |
| TLR7(rs1620233) | C>T | 0.660 | 100.00 | 0.034 | 0.045 | 0.063 |
| TLR7(rs179012) | G>A | 0.742 | 100.00 | 0.092 | 0.095 | 0.127 |
| TLR7(rs179009) | A>G | 0.576 | 100.00 | 0.181 | 0.180 | 0.135 |
| TLR7(rs179010) | C>T | 0.571 | 99.84 | 0.353 | 0.349 | 0.349 |
| TLR7(rs179019) | C>A | 0.459 | 100.00 | 0.257 | 0.243 | 0.278 |
| TLR7(rs1634322) | T>A | 0.778 | 100.00 | 0.124 | 0.136 | 0.135 |
| TLR7(rs5743740) | A>G | 0.772 | 100.00 | 0.124 | 0.148 | 0.175 |
| TLR7(rs5741880) | G>T | 0.054 | 100.00 | 0.057 | 0.037 | 0.040 |
| TLR8(rs2159377) | T>C | 0.160 | 100.00 | 0.246 | 0.254 | 0.262 |
| TLR8(rs17256081) | T>C | 0.122 | 100.00 | 0.170 | 0.153 | 0.164 |
| TLR8(rs4830805) | A>G | 0.483 | 100.00 | 0.200 | 0.193 | 0.230 |
| TLR8(rs5744068) | C>T | 0.782 | 100.00 | 0.025 | 0.026 | 0.063 |
| TLR8(rs3747414) | A>C | 0.211 | 100.00 | 0.218 | 0.220 | 0.183 |
| TLR8(rs5741883) | C>T | 0.782 | 100.00 | 0.025 | 0.019 | 0.056 |

Abbreviations: SNPs, single nucleotide polymorphisms; HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency.

Similar associations were also seen in female asthmatics (Table 4). Noteworthily, the associations in males and females were different; for instance, SNP rs1620233 was associated with eosinophil counts in female but not male asthmatics, yet SNPs (rs179009, rs179010, rs1634322, and rs4830805) were associated with eosinophil counts only in male asthmatics. And the association between SNP rs17256081 and asthma severity was found only in female asthmatics.

**Table 3. Association of *TLR7/8* polymorphisms with asthma-related phenotypes in males**

| SNPs | Eos ($\times 10^6$/ml) | $FEV_1$% (%) | $FEV_1$ / FVC (%) | IgE (IU/ml) | Asthma Severity *n* (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Stage 1 | Stage 2 | Stage 3 | Stage 4 |
| rs1620233 | | | | | | | | |
| C | 0.60±1.48 | 64.85±23.10 | 65.72±15.53 | 136.15±133.07 | 54(34.62) | 42(26.92) | 26(16.67) | 34(21.79) |
| T | 0.45±0.05 | 67.12±20.89 | 66.65±19.56 | 216.75±235.85 | 0(0.00) | 4(50.00) | 4(50.00) | 0(0.00) |
| $P_{FDR}$ | 0.366 | 0.795 | 0.906 | 0.029 | 0.803 | | | |
| rs179012 | | | | | | | | |
| G | 0.63±1.52 | 60.98±22.67 | 64.97±16.79 | 146.63±142.32 | 52(33.77) | 42(27.27) | 30(19.48) | 30(19.48) |
| A | 0.28±0.06 | 86.10±11.66 | 69.85±13.29 | 64.17±40.76 | 2(20.00) | 4(40.00) | 0(0.00) | 4(40.00) |
| $P_{FDR}$ | 0.431 | <0.001 | 0.004 | 0.005 | 0.789 | | | |
| rs179009 | | | | | | | | |
| A | 0.32±0.27 | 62.13±23.30 | 65.39±16.51 | 128.83±118.59 | 44(33.33) | 38(28.79) | 22(16.67) | 28(21.21) |
| G | 1.26±2.58 | 79.80±17.09 | 67.50±9.81 | 169.44±180.56 | 10(31.25) | 8(25.00) | 8(25.00) | 6(18.75) |
| $P_{FDR}$ | 0.035 | 0.383 | 0.906 | 0.977 | 0.917 | | | |
| rs179010 | | | | | | | | |
| C | 0.67±1.62 | 64.02±23.26 | 64.26±14.74 | 152.01±144.96 | 38(33.33) | 32(28.07) | 22(19.30) | 22(19.30) |
| T | 0.32±0.24 | 66.16±24.02 | 68.05±17.27 | 94.85±103.54 | 16(32.00) | 14(28.00) | 8(16.00) | 12(24.00) |
| $P_{FDR}$ | 0.035 | 0.644 | 0.002 | 0.540 | 0.917 | | | |

**Table 3. Continue**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| rs179019 | | | | | | | | |
| C | 0.65±1.60 | 65.80±22.45 | 65.46±14.27 | 150.36±142.28 | 42(32.81) | 36(28.13) | 26(20.31) | 24(18.75) |
| A | 0.37±0.22 | 62.70±25.95 | 66.31±19.14 | 84.87±106.53 | 12(33.33) | 10(27.78) | 4(11.11) | 10(27.78) |
| $P_{FDR}$ | 0.653 | 0.818 | 0.906 | 0.312 | 0.917 | | | |
| rs1634322 | | | | | | | | |
| T | 0.62±1.50 | 63.58±23.63 | 64.94±15.94 | 136.80±135.18 | 50(34.25) | 36(24.66) | 26(17.81) | 34(23.29) |
| A | 0.22±0.26 | 65.45±21.55 | 62.02±16.21 | 170.78±175.24 | 4(22.22) | 10(55.56) | 4(22.22) | 0(0.00) |
| $P_{FDR}$ | 0.035 | 0.538 | 0.695 | 0.556 | 0.789 | | | |
| rs5743740 | | | | | | | | |
| A | 0.62±1.52 | 62.23±22.08 | 65.14±16.05 | 141.67±134.71 | 52(36.11) | 38(26.39) | 24(16.67) | 30(20.83) |
| G | 0.36±0.07 | 76.20±13.69 | 72.70±11.01 | 128.20±181.54 | 2(10.00) | 8(40.00) | 6(30.00) | 4(20.00) |
| $P_{FDR}$ | 0.730 | 0.827 | 0.001 | 0.818 | 0.400 | | | |
| rs5741880 | | | | | | | | |
| G | 0.62±1.47 | 64.85±23.10 | 65.72±15.53 | 143.02±139.62 | 52(32.91) | 42(26.58) | 30(18.99) | 34(21.52) |
| T | 0.55±1.69 | 69.43±20.17 | 63.75±17.37 | 103.02±123.76 | 2(33.33) | 4(66.67) | 0(0.00) | 0(0.00) |
| $P_{FDR}$ | 0.874 | 0.502 | 0.874 | 0.556 | 0.731 | | | |
| rs2159377 | | | | | | | | |
| T | 0.71±1.68 | 69.36±22.37 | 70.52±12.65 | 150.52±147.60 | 40(32.26) | 36(29.03) | 24(19.35) | 24(19.35) |
| C | 0.30±0.23 | 49.80±20.30 | 49.71±14.10 | 110.61±107.27 | 14(35.00) | 10(25.00) | 6(15.00) | 10(25.00) |
| $P_{FDR}$ | 0.035 | 0.037 | 0.007 | 0.114 | 0.917 | | | |
| rs17256081 | | | | | | | | |
| T | 0.67±1.56 | 63.92±23.84 | 65.61±16.19 | 139.73±143.44 | 48(34.29) | 38(27.14) | 26(18.57) | 28(20.00) |
| C | 0.15±0.21 | 65.00±22.86 | 67.07±14.56 | 143.33±115.50 | 6(25.00) | 8(33.33) | 4(16.67) | 6(25.00) |
| $P_{FDR}$ | 0.035 | 0.644 | 0.906 | 0.501 | 0.789 | | | |
| rs4830805 | | | | | | | | |
| A | 0.73±1.22 | 70.13±21.39 | 72.12±13.16 | 123.51±127.63 | 46(35.38) | 36(27.69) | 24(18.46) | 24(18.46) |
| G | 0.31±0.35 | 59.71±22.12 | 53.32±15.17 | 111.64±121.09 | 8(23.53) | 10(29.41) | 6(17.65) | 10(29.41) |
| $P_{FDR}$ | 0.035 | 0.091 | 0.042 | 0.556 | 0.400 | | | |
| rs5744068 | | | | | | | | |
| C | 0.60±1.53 | 67.15±22.54 | 68.18±13.41 | 149.94±140.20 | 50(32.47) | 44(28.57) | 28(18.18) | 32(20.78) |
| T | 0.54±0.02 | 47.20±21.03 | 46.14±12.19 | 24.47±16.18 | 4(40.00) | 2(20.00) | 2(20.00) | 2(20.00) |
| $P_{FDR}$ | 0.601 | 0.217 | 0.008 | 0.055 | 0.917 | | | |
| rs3747414 | | | | | | | | |
| A | 0.69±1.62 | 69.11±22.73 | 67.16±16.50 | 133.49±144.62 | 46(34.85) | 38(28.79) | 24(18.18) | 24(18.18) |
| C | 0.24±0.23 | 62.63±19.71 | 65.90±11.60 | 166.60±113.86 | 8(25.00) | 8(25.00) | 6(18.75) | 10(31.25) |
| $P_{FDR}$ | 0.331 | 0.466 | 0.695 | 0.501 | 0.400 | | | |
| rs5741883 | | | | | | | | |
| C | 0.60±1.50 | 67.15±22.54 | 68.18±13.41 | 146.23±140.07 | 54(34.18) | 44(27.85) | 28(17.72) | 32(20.25) |
| T | 0.56±0.01 | 65.20±23.02 | 66.14±12.54 | 30.30±17.32 | 0(0.00) | 2(33.33) | 2(33.33) | 2(33.33) |
| $P_{FDR}$ | 0.609 | 0.844 | 0.906 | 0.052 | 0.400 | | | |

Abbreviations: Eos, eosinophils; $FEV_1$%, percent of predicted forced expiratory volume in 1 second; $FEV_1$/FVC: $FEV_1$ as percentage of forced vital capacity; FDR, false-discovery rate; IgE, immunoglobulin E levels; SNPs, single nucleotide polymorphisms.

Stage 1: intermittent; Stage 2: mild-persistent; Stage 3: moderate-persistent; Stage 4: severe-persistent.

**Table 4. Association of TLR7/8 polymorphisms with asthma-related phenotypes in females**

| SNPs | Eos ($\times 10^6$/ml) | $FEV_1$% (%) | $FEV_1$ / FVC (%) | IgE (IU/ml) | Asthma Severity *n* (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Stage 1 | Stage 2 | Stage 3 | Stage 4 |
| rs1620233 | | | | | | | | |
| CC | 0.22±0.24 | 77.43±18.37 | 70.94±14.48 | 140.84±143.17 | 74(27.61) | 94(35.07) | 38(14.18) | 62(23.13) |
| CT | 0.32±0.30 | 75.49±17.69 | 72.53±15.99 | 55.22±46.67 | 6(25.00) | 4(16.67) | 8(33.33) | 6(25.00) |
| TT | - | - | - | - | - | - | - | - |
| $P_{FDR}$ | 0.036 | 0.689 | 0.810 | 0.148 | 0.545 | | | |
| rs179012 | | | | | | | | |
| GG | 0.21±0.22 | 73.54±21.01 | 67.57±16.82 | 140.03±148.56 | 60(25.42) | 80(33.90) | 42(17.80) | 54(22.88) |
| GA | 0.36±0.42 | 76.22±18.21 | 70.25±15.68 | 110.13±65.41 | 20(40.00) | 14(28.00) | 4(8.00) | 12(24.00) |
| AA | 0.21±0.05 | 75.15±20.23 | 71.65±17.62 | 74.15±58.72 | 0(0.00) | 4(66.67) | 0(0.00) | 2(33.33) |
| $P_{FDR}$ | 0.292 | 0.689 | 0.774 | 0.213 | 0.557 | | | |
| rs179009 | | | | | | | | |
| AA | 0.23±0.24 | 74.91±23.02 | 66.42±19.24 | 133.62±132.31 | 52(26.53) | 68(34.69) | 32(16.33) | 44(22.45) |
| AG | 0.20±0.25 | 73.54±20.14 | 71.87±13.06 | 128.05±150.74 | 26(28.26) | 30(32.61) | 14(15.22) | 22(23.91) |
| GG | 0.26±0.20 | 76.53±22.46 | 72.83±16.07 | 130.11±147.56 | 2(50.00) | 0(0.00) | 0(0.00) | 2(50.00) |
| $P_{FDR}$ | 0.626 | 0.689 | 0.774 | 0.822 | 0.908 | | | |
| rs179010 | | | | | | | | |
| CC | 0.21±0.20 | 72.37±24.77 | 68.05±22.14 | 140.76±145.46 | 36(26.47) | 52(38.24) | 14(10.29) | 34(25.00) |
| CT | 0.24±0.29 | 80.43±19.14 | 72.17±9.08 | 122.54±137.70 | 28(28.00) | 34(34.00) | 18(18.00) | 20(20.00) |
| TT | 0.23±0.23 | 66.55±17.79 | 61.84±21.97 | 144.15±137.07 | 16(28.57) | 12(21.43) | 14(25.00) | 14(25.00) |
| $P_{FDR}$ | 0.152 | 0.168 | 0.774 | 0.542 | 0.770 | | | |
| rs179019 | | | | | | | | |
| CC | 0.18±0.19 | 76.84±22.27 | 66.64±20.45 | 131.93±158.90 | 48(29.63) | 56(34.57) | 20(12.35) | 38(23.46) |
| CA | 0.28±0.29 | 72.39±20.29 | 70.99±12.48 | 121.18±113.95 | 28(24.56) | 38(33.33) | 20(17.54) | 28(24.56) |
| AA | 0.11±0.11 | 72.39±20.29 | 71.04±10.46 | 120.69±18.14 | 4(25.00) | 4(25.00) | 6(37.50) | 2(12.50) |
| $P_{FDR}$ | 0.068 | 0.288 | 0.073 | 0.542 | 0.720 | | | |
| rs1634322 | | | | | | | | |
| TT | 0.24±0.26 | 78.15±20.62 | 69.61±12.42 | 138.92±139.03 | 52(24.76) | 78(37.14) | 30(14.29) | 50(23.81) |
| TA | 0.21±0.22 | 72.12±22.14 | 67.44±22.34 | 125.93±146.80 | 28(35.00) | 20(25.00) | 16(20.00) | 16(20.00) |
| AA | 0.21±0.19 | 68.12±16.52 | 68.32±15.69 | 145.30±150.78 | 0(0.00) | 0(0.00) | 0(0.00) | 2(100.00) |
| $P_{FDR}$ | 0.465 | 0.221 | 0.810 | 0.873 | 0.105 | | | |
| rs5743740 | | | | | | | | |
| AA | 0.21±0.21 | 77.07±17.06 | 69.72±13.96 | 144.15±150.42 | 62(29.25) | 70(33.02) | 34(16.04) | 46(21.70) |
| AG | 0.24±0.36 | 68.14±30.87 | 65.82±24.48 | 114.19±93.18 | 14(20.00) | 26(37.14) | 12(17.14) | 18(25.71) |
| GG | 0.34±0.19 | 65.56±19.12 | 68.14±17.23 | 122.30±120.15 | 4(40.00) | 2(20.00) | 0(0.00) | 4(40.00) |
| $P_{FDR}$ | 0.068 | 0.168 | 0.810 | 0.324 | 0.547 | | | |
| rs5741880 | | | | | | | | |
| GG | 0.22±0.25 | 74.17±22.26 | 67.48±17.31 | 130.20±137.03 | 72(26.67) | 94(34.81) | 44(16.30) | 60(22.22) |
| GT | 0.23±0.20 | 80.80±6.00 | 79.05±11.60 | 171.10±163.71 | 8(36.36) | 4(18.18) | 2(9.09) | 8(36.36) |
| TT | - | - | - | - | - | - | - | - |
| $P_{FDR}$ | 0.540 | 0.689 | 0.774 | 0.213 | 0.840 | | | |
| rs2159377 | | | | | | | | |
| TT | 0.22±0.27 | 71.90±20.93 | 71.40±13.19 | 122.07±133.19 | 40(24.10) | 50(30.12) | 32(19.28) | 44(26.51) |
| TC | 0.21±0.21 | 79.04±23.46 | 69.71±18.96 | 137.79±146.45 | 34(33.33) | 32(31.37) | 14(13.73) | 22(21.57) |
| CC | 0.30±0.22 | 69.10±8.20 | 52.50±20.21 | 221.14±145.10 | 6(25.00) | 16(66.67) | 0(0.00) | 2(8.33) |

**Table 4. Continue**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| $P_{FDR}$ | 0.036 | 0.271 | 0.001 | 0.822 | 0.105 | | | |
| rs17256081 | | | | | | | | |
| TT | 0.24±0.25 | 67.51±19.64 | 69.45±15.25 | 145.80±149.70 | 54(24.77) | 68(31.19) | 40(18.35) | 56(25.69) |
| TC | 0.19±0.24 | 75.10±29.89 | 63.60±27.66 | 105.80±97.65 | 18(32.14) | 24(42.86) | 4(7.14) | 10(17.86) |
| CC | 0.17±0.13 | 80.70±18.12 | 65.66±23.45 | 110.68±155.71 | 8(44.44) | 6(33.33) | 2(11.11) | 2(11.11) |
| $P_{FDR}$ | 0.003 | 0.032 | 0.810 | 0.148 | 0.037 | | | |
| rs4830805 | | | | | | | | |
| AA | 0.24±0.21 | 68.74±19.76 | 70.41±15.34 | 151.24±152.65 | 46(23.23) | 64(32.32) | 36(18.18) | 52(26.26) |
| AG | 0.19±0.22 | 78.52±23.92 | 61.70±23.64 | 101.01±96.09 | 26(34.21) | 28(36.84) | 8(10.53) | 14(18.42) |
| GG | 0.20±0.13 | 79.70±17.23 | 72.70±0.00 | 110.68±155.71 | 8(44.44) | 6(33.33) | 2(11.11) | 2(11.11) |
| $P_{FDR}$ | 0.065 | 0.032 | 0.774 | 0.324 | 0.093 | | | |
| rs5744068 | | | | | | | | |
| CC | 0.22±0.24 | 74.76±21.82 | 68.62±17.54 | 131.55±139.36 | 78(27.66) | 92(32.62) | 44(15.60) | 68(24.11) |
| CT | 0.25±0.23 | 76.20±19.08 | 70.00±19.21 | 217.30±141.22 | 2(20.00) | 6(60.00) | 2(20.00) | 0(0.00) |
| TT | - | - | - | - | - | - | - | - |
| $P_{FDR}$ | 0.772 | 0.689 | 0.810 | 0.213 | 0.572 | | | |
| rs3747414 | | | | | | | | |
| AA | 0.22±0.26 | 72.66±20.46 | 71.51±13.77 | 117.94±130.44 | 46(26.14) | 50(28.41) | 34(19.32) | 46(26.14) |
| AC | 0.24±0.22 | 79.42±23.18 | 69.09±18.27 | 151.35±151.85 | 28(28.00) | 42(42.00) | 10(10.00) | 20(20.00) |
| CC | 0.15±0.17 | 72.08±21.14 | 66.89±15.84 | 149.10±72.75 | 6(37.50) | 6(37.50) | 2(12.50) | 2(12.50) |
| $P_{FDR}$ | 0.056 | 0.271 | 0.774 | 0.822 | 0.344 | | | |
| rs5741883 | | | | | | | | |
| CC | 0.22±0.24 | 74.83±21.25 | 68.70±17.06 | 134.81±140.23 | 78(27.46) | 96(33.80) | 44(15.49) | 66(23.24) |
| CT | 0.23±0.21 | 71.22±20.89 | 70.45±19.24 | 158.45±136.78 | 2(25.00) | 2(25.00) | 2(25.00) | 2(25.00) |
| TT | - | - | - | - | - | - | - | - |
| $P_{FDR}$ | 0.465 | 0.689 | 0.810 | 0.822 | 0.840 | | | |

Abbreviations: Eos, eosinophils; $FEV_1$%, percent of predicted forced expiratory volume in 1 second; $FEV_1$/FVC: $FEV_1$ as percentage of forced vital capacity; FDR, false-discovery rate; IgE, immunoglobulin E levels; SNPs, single nucleotide polymorphisms.

Stage 1: intermittent; Stage 2: mild-persistent; Stage 3: moderate-persistent; Stage 4: severe-persistent.

## DISCUSSION

In the present study, we investigated the possible role of fourteen haplotype tagging SNPs, capturing all essential genetic information of the TLR7/TLR8 gene locus, on asthma susceptibility and asthma-related phenotypes in a Chinese population. Our results indicated that the TLR7/TLR8 SNPs were not associated with the risk of asthma. However, we found that the genetic variants in TLR7/TLR8 had influences on asthma-related phenotypes, including eosinophil counts, total serum IgE levels, lung function, and asthma severity.

TLR7 and TLR8, with homologous function, link innate and adaptive immunity and mediate anti-virus immunity, by producing Th1-directed responses including type I interferon.[30] Our previous study[31] demonstrated that TLR7 agonist prevented Th2-mediated airway inflammation in an ovalbumin-induced asthmatic murine model, which was consistent with the other findings.[11-13,32] The effects of TLR7 signaling are not limited to animal models of Th2-directed inflammation. Human peripheral mononuclear cells produced decreased IgE in response to R848, a synthetic TLR7/8 agonist, in an IFN-γ-dependent manner in vitro,[33] and Th2 cells were reduced and Th1 cells were increased after treatment with R848.[34] Furthermore, it was reported in recent studies that TLR7 function was reduced in adolescents with asthma,[35] and interferon induced by rhinovirus (natural ligand of TLR7/8) was deficient in asthmatic patients but not in well controlled asthmatics.[36,37] These data

highlight that TLR7 and TLR8 play a critical role in the pathogenesis of asthma.

Our previous studies revealed the role of TLR4[38] and TLR2[39] subfamily gene polymorphisms on asthma in a Chinese population. In the present study, we found the TT genotype of rs179010 in TLR7 was over-represented in female asthmatics, when compared with controls; however, these associations were not significant after correcting for multiple comparisons. Our findings were inconsistent with the study by Møller-Larsen et al.,[23] which suggested the SNPs in TLR7/8 showed significant associations with asthma. We think this may be due to the genetic differences of race and the crowd, because different genetic background and inheritance patterns have different effects on disease. In addition, different living environments (including natural environments and social environments), and the strength of the interaction between genes and environment exercise a great influence on disease phenotype. It is important to note that our previous positive report[24] could be a false positive, because no correction for multiple tests was applied.

It is not surprising that significant associations were seen in the present study between the SNPs in TLR7/8 and eosinophil counts and total serum IgE levels. Human eosinophils constitutively express high level of TLR7.[40] Stimulation of TLR7 was found to prolong survival and activation of eosinophils.[41] Although TLR8 expression was hardly detectable in freshly isolated eosinophils, it was exclusively up-regulated by IFN-γ and maybe also regulated the function of eosinophils.[42] Moreover, Stimulation of murine B cells with TLR7/8 ligand R848 induced inhibition of IgE and IgG1 synthesis.[43] In contrast, human B cells proliferated and turned into antibody-secreting cells in response to TLR7 ligand, producing IgG1, IgA, IgG4 and very low levels of IgE.[44] Although a discrepancy in these two reports existed, the modulation of IgE synthesis by TLR7 was evident. Most notably, our results underscored that the presence of TLR7/8 polymorphisms may influence lung function and asthma severity. It is well known that viral respiratory infections, especially with rhinovirus, are a major cause of asthma exacerbations and induce more frequent and longer lasting lower respiratory tract symptoms. TLR7 function is reduced in adolescents with asthma and this may contribute to susceptibility to respiratory viral infections.[35] Moreover, in addition to the immunoregulatory potential, TLR7 can mediate relaxation of human and animal airways through nitric oxide production while TLR8 executes relaxant effects via nitric oxide-independent pathway.[16,45] Thus, we hypothesize that genetic variants may alter the function of TLR7/8, and consequently affect the lung function and severity of asthma. Interestingly, we observed that the associations between TLR7/8 polymorphisms in males and females were different, which demonstrated a sex-specific effect. Actually, several investigations also showed sex-specific association between TLR7 and human diseases.[46,47] The exact gender-dependent mechanisms are not yet clear. This differential influence on diverse sex 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 presented in males, accounting for the consequence of genetic variation and the immunobiology of TLR7/8.[48] Additionally, the gender-specific differences in hormone secretions, physiological functions, socio-cultural attitudes, and lifestyle exposures to environment should be taken into consideration.[49]

In the present study, asthma and asthma-related phenotypes were classified following restrictive criteria, which, in theory, should limit possible confounding effects. In addition, we selected haplotype tagging SNPs from the HapMap database, which captured all essential genetic information of the TLR7/8 gene locus, avoiding missing other potentially functional polymorphisms. Nevertheless, our study also had some limitations. First, sample size was relatively small, especially stratified into males and females, which may be prone to false negatives due to low statistical power. Second, environmental factors other than smoking (e.g., occupational exposure, pets, and allergen exposure) might interact with the TLR7/8 genotype or act as potential confounders in the analysis.

In summary, our study suggests that the polymorphisms in TLR7/8 are associated with asthma-related phenotypes but not asthma risk in the study population. We believe that this work will help in better understanding the pathogenesis of asthma and development of more effective strategies for asthma prevention, prediction, and therapy. Further investigation on TLR7/8 as a drug target in asthma is attractive.

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**REFERENCES**

1. Busse WW, Lemanske RF Jr. Asthma. N Engl J Med 2001; 344(5):350-62.
2. Akhabir L, Sandford AJ. Genome-wide association studies for discovery of genes involved in asthma. Respirology 2011; 16(3):396-406.
3. Romagnani S. The increased prevalence of allergy and the hygiene hypothesis: missing immune deviation, reduced immune suppression, or both? Immunology 2004; 112(3):352-63.
4. Iwasaki A, Medzhitov R. Regulation of adaptive immunity by the innate immune system. Science 2010; 327(5963):291-5.
5. Barton GM, Medzhitov R. Toll-like receptor signaling pathways. Science 2003; 300(5625):1524-5.
6. Sun J, Walsh M, Villarino AV, Cervi L, Hunter CA, Choi Y, et al. TLR ligands can activate dendritic cells to provide a MyD88-dependent negative signal for Th2 cell development. J Immunol 2005; 174(2):742-51.
7. Conroy H, Marshall NA, Mills KH. TLR ligand suppression or enhancement of Treg cells? A double-edged sword in immunity to tumours. Oncogene 2008; 27(2):168-80.
8. Cherfils-Vicini J, Platonova S, Gillard M, Laurans L, Validire P, Caliandro R, et al. Triggering of TLR7 and TLR8 expressed by human lung cancer cells induces cell survival and chemoresistance. J Clin Invest 2010; 120(4):1285-97.
9. Ekman AK, Adner M, Cardell LO. Toll-like receptor 7 activation reduces the contractile response of airway smooth muscle. Eur J Pharmacol 2011; 652(1-3):145-51.
10. Heil F, Hemmi H, Hochrein H, Ampenberger F, Kirschning C, Akira S, et al. Species-specific recognition of single-stranded RNA via Toll-like receptor 7 and 8. Science 2004; 303(5663):1526-9.
11. Xirakia C, Koltsida O, Stavropoulos A, Thanassopoulou A, Aidinis V, Sideras P, at 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.
12. 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.
13. 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.
14. Edwards S, Jones C, Leishman AJ, Young BW, Matsui H, Tomizawa H, et al. TLR7 stimulation of APCs results in inhibition of IL-5 through type I IFN and Notch signaling pathways in human peripheral blood mononuclear cells. J Immunol 2013; 190(6):2585-92.
15. Greiff L, Cervin A, Ahlstrom-Emanuelsson C, Almqvist G, Andersson M, Dolata J, et al. Repeated intranasal TLR7 stimulation reduces allergen responsiveness in allergic rhinitis. Respir Res 2012; 13:53.
16. Drake MG, Scott GD, Proskocil BJ, Fryer AD, Jacoby DB, Kaufman EH. Toll-like receptor 7 rapidly relaxes human airways. Am J Respir Crit Care Med 2013; 188(6):664-72.
17. Chuang TH, Ulevitch RJ. Cloning and characterization of a sub-family of human toll-like receptors: hTLR7, hTLR8 and hTLR9. Eur Cytokine Netw 2000; 11(3):372-8.
18. Enevold C, Nielsen CH, Jacobsen RS, Hermansen ML, Molbo D, Avlund K, et al. Single nucleotide polymorphisms in genes encoding toll-like receptors 7, 8 and 9 in Danish patients with systemic lupus erythematosus. Mol Biol Rep 2014; 41(9):5755-63.
19. Wang CH, Eng HL, Lin KH, Liu HC, Chang CH, Lin TM. Functional polymorphisms of TLR8 are associated with hepatitis C virus infection. Immunology 2014; 141(4):540-8.
20. Sada T, Ota M, Katsuyama Y, Meguro A, Nomura E, Uemoto R, et al. Association analysis of Toll-like receptor 7 gene polymorphisms and Behçet's disease in Japanese patients. Hum Immunol 2011; 72(3):269-72.
21. Oh DY, Baumann K, Hamouda O, Eckert JK, Neumann K, Kücherer C, et al. A frequent functional toll-like receptor 7 polymorphism is associated with accelerated HIV-1 disease progression. AIDS 2009; 23(3):297-307.

22. Nilsson D, Andiappan AK, Halldén C, De Yun W, Säll T, Tim CF, et al. Toll-like receptor gene polymorphisms are associated with allergic rhinitis: a case control study. BMC Med Genet 2012; 13:66.
23. 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.
24. Zhang Q, Qian FH, Zhou LF, Wei GZ, Wang Y, Hu ZB, et al. Polymorphisms of TLR7 and TLR8 associated with risk of asthma and asthma-related phenotypes in a southeastern Chinese Han population. Journal of Nanjing Medical University 2009; 23(1):25-32.
25. The Global Strategy for Asthma Management and Prevention. Global Initiative for Asthma (GINA) 2010. Available from: http://www.ginasthma.org/.
26. Wu X, Gu J, Grossman HB, Amos CI, Etzel C, Huang M, et al. Bladder cancer predisposition: a multigenic approach to DNA-repair and cell-cycle-control genes. Am J Hum Genet 2006; 78(3):464-79.
27. Qian FH, Zhang Q, Zhou LF, Liu H, Huang M, Zhang XL, et al. High sensitivity C-reactive protein: a predicative marker in severe asthma. Respirology 2008; 13(5):664-9.
28. Hu XP, Wu JQ, Zhu LP, Wang X, Xu B, Wang RY, et al. Association of Fcγ receptor IIB polymorphism with cryptococcal meningitis in HIV-uninfected Chinese patients. PLoS One 2012; 7(8):e42439.
29. Esteves LM, Bulhões SM, Brilhante MJ, Mota-Vieira L. Three multiplex snapshot assays for SNP genotyping in candidate innate immune genes. BMC Res Notes 2013; 6:54.
30. Yang K, Puel A, Zhang S, Eidenschenk C, Ku CL, Casrouge A, et al. Human TLR-7-, -8-, and -9-mediated induction of IFN-alpha/beta and -lambda is IRAK-4 dependent and redundant for protective immunity to viruses. Immunity 2005; 23(5):465-78.
31. Du Q, Zhou LF, Chen Z, Gu XY, Huang M, Yin KS. Imiquimod, a toll-like receptor 7 ligand, inhibits airway remodelling in a murine model of chronic asthma. Clin Exp Pharmacol Physiol 2009; 36(1):43-8.
32. 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.
33. Frotscher B, Anton K, Worm M. Inhibition of IgE production by the imidazoquinoline resiquimod in nonallergic and allergic donors. J Invest Dermatol 2002; 119(5):1059-64.
34. Filì L, Ferri S, Guarna F, Sampognaro S, Manuelli C, Liotta F, et al. Redirection of allergen-specific TH2 responses by a modified adenine through Toll-like receptor 7 interaction and IL-12/IFN release. J Allergy Clin Immunol 2006; 118(2):511-7.
35. 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.
36. 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.
37. 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.
38. Zhang Q, Qian FH, Zhou LF, Wei GZ, Jin GF, Bai JL, et al. Polymorphisms in toll-like receptor 4 gene are associated with asthma severity but not susceptibility in a Chinese Han population. J Investig Allergol Clin Immunol 2011; 21(5):370-7.
39. Qian FH, Zhang Q, Zhou LF, Jin GF, Bai JL, Yin KS. Polymorphisms in the toll-like receptor 2 subfamily and risk of asthma: a case-control analysis in a Chinese population. J Investig Allergol Clin Immunol 2010; 20(4):340-6.
40. Kvarnhammar AM, Cardell LO. Pattern-recognition receptors in human eosinophils. Immunology 2012; 136(1):11-20.
41. Månsson A, Cardell LO. Role of atopic status in Toll-like receptor (TLR)7- and TLR9-mediated activation of human eosinophils. J Leukoc Biol 2009; 85(4):719-27.
42. Nagase H, Okugawa S, Ota Y, Yamaguchi M, Tomizawa H, Matsushima K, et al. Expression and function of Toll-like receptors in eosinophils: activation by Toll-like receptor 7 ligand. J Immunol 2003; 171(8):3977-82.
43. Shen E, Lu L, Wu C. TLR7/8 ligand, R-848, inhibits IgE synthesis by acting directly on B lymphocytes. Scand J Immunol 2008; 67(6):560-8.
44. Sackesen C, van de Veen W, Akdis M, Soyer O, Zumkehr J, Ruckert B, et al. Suppression of B-cell activation and IgE, IgA, IgG1 and IgG4 production by mammalian telomeric oligonucleotides. Allergy 2013; 68(5):593-603.

45. Kaufman EH, Fryer AD, Jacoby DB. Toll-like receptor 7 agonists are potent and rapid bronchodilators in guinea pigs. J Allergy Clin Immunol 2011; 127(2):462-9.
46. 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.
47. 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.
48. 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.
49. Osman M, Hansell AL, Simpson CR, Hollowell J, Helms PJ. Gender-specific presentations for asthma, allergic rhinitis and eczema in primary care. Prim Care Respir J 2007; 16(1):28-35.