International Journal of Biological Sciences
2009; 5(1):74-81

Research Paper

STAT2*C related genotypes and allele but not TLR4 and CD40 gene polymorphisms are associated with higher susceptibility for asthma

Yao-Yuan Hsieh[1,3*], Lei Wan[2,3,4*], Chi-Chen Chang[1], Chang-Hai Tsai[2,4], Fuu-Jen Tsai[2,3,4] ✉

1. Department of Obstetrics and Gynecology, China Medical University Hospital, Taichung, Taiwan
2. Department of Medical Research, China Medical University Hospital, Taichung, Taiwan
3. Graduate Institute of Chinese Medical Science, China Medical University, Taichung, Taiwan
4. Department of Biotechnology, Asia University, Taichung, Taiwan

[*] Yao-Yuan Hsieh and Lei Wan contribute equally to this survey

✉ Correspondence to: Fuu-Jen Tsai, M.D., Ph.D., Department of Pediatrics and Medical Genetics, China Medical University Hospital, No.2 Yuh-Der Road, Taichung, Taiwan. Telephone: 886-4-22052121 ext. 2041, Fax: 886-4-22033295, E-mail: d0704@mail.cmuh.org.tw

Received: 2008.12.14; Accepted: 2009.01.08; Published: 2009.01.09

Abstract

Objective: Asthma is caused by a complex interaction between multiple genes and environmental factors. Herein we aimed to investigate whether signal transducer and activator of transcription (STAT2), toll-like receptors 4 (TLRs4) and CD40-related polymorphisms are associated with asthma susceptibility.

Design: Children were divided: (1) asthma (n=117); (2) normal controls (n=60). The polymorphisms of STAT2, TLR4 and CD40 polymorphism were analyzed by PCR-RFLP genotyping. Genotypes, allelic frequencies and association of haplotypes in both groups were compared.

Results: STAT2*C related genotypes, but not TLR4 and CD40 polymorphism, are associated with higher susceptibility for asthma. Distributions of STAT2*CC/CG/GG and C/G allele in both groups are: (1) 0/11.1/88.9 % and 5.6/94.4%; (2) 0/1.7/98.3% and 0.8/99.2% ($p<0.05$). Proportions of TLR4*rs10983755 AA/AG/GG and rs1927914 CC/CT/TT homozygote are: (1) 35.1/8.5/56.4% and 9.4/56.4/34.2%; (2) 35/8.3/56.7% and 16.7/48.3/35% (non-difference). Proportions of CD40*rs1883832 CC/CT/TT, rs3765459 AA/AG/GG, and rs4810485 TT/GT/GG are: (1) 29.9/53/17.1%, 6.8/47.9/45.3 and 18.8/62.4/18.8%; (2) 36.7/41.7/21.6%, 1.6/46.7/ 51.7 and 15/51.7/33.3% (non-difference). Haplotype analyses for TLR4 and CD40 genes revealed their non-association and non-additional effect upon asthma susceptibilities.

Conclusion: STAT2*C related genotypes and alleles are associated with asthma susceptibilities and pathogenesis. There were non-association and non-additional effects of TLR4/CD40 gene polymorphisms and haplotypes upon asthma risk.

Key words: Asthma, CD40, polymorphism, SNP, STAT2, TLR4

Introduction

Asthma, one major respiratory consequence, appeared around 6-9% prevalence in general population [1]. The incidence of asthma appeared the increased trend during past decade. Asthma is caused by a complex interaction between multiple candidate genes and environmental factors. However, the related molecular basis for this upper airway disorder remains unclear. The raised incidence of asthma has

been attributed to increased environment contamination, overusage of antibiotics as well as constitutional and genetic factors. However, the mechanistic roles of the disease-associated SNPs have yet to be elucidated especially in the context of the pathophysiology of asthma. Furthermore, the related factor for predicting asthma susceptibilities remains obscure.

Numerous cytokines play an important role in allergic immune disorders, such as asthma. Proinflammatory cytokines could contribute to this inflammatory process for asthma [2]. These cytokines regulate diverse biological functions by binding to receptors at the cell surface to activate complex signal transduction pathways, including the signal transducer and activator of transcription (STAT) signaling pathways. The STAT pathway mediates the signals of a wide range of cytokines, growth factors and hormones. Aberrant activation of STAT pathway may predispose to cell dysfunction or dysregulation. Signal transducer and activator of transcription (STAT) has been demonstrated to be associated with asthma susceptibilities [3]. Some STAT gene variation (C39134A) might be associated with IgE regulation and atopy [4]. .

Infectious diseases have a major impact on both the development and severity of asthma. Innate immunity status and related genetic variations have been reported to be associated with inflammatory disorders such as asthma. Toll-like receptors (TLRs) are involved in immune responses towards various micro-organisms. TLRs are innate immune sensors of microbial cell wall products that initiate early host responses [5]. TLRs play a pivotal role in the induction of first-line defense mechanisms of the immune system and trigger adaptive immune responses to microbial pathogens.

The CD40 protein plays important roles in cell-mediated and humoral immune responses [6]. CD40 protein is expressed in a variety of cell types. CD40 ligation causes cells to produce inflammatory cytokines and cellular adhesion molecules. CD40 gene polymorphisms exert a genetic effect on IgE production in patients with asthma through translational regulation of CD40 expression on B cells [6]. Asthma is characterized by airway smooth muscle hyperplasia, inflammatory cell infiltration, and increased expression of cytokines. These cytokines have the potential to alter the expression of surface receptors such as CD40 ligand on the airway smooth muscle cell [7].

Reviewing MEDLINE database, few investigator demonstrated the correlation of STAT2, TLR4 and CD40 gene polymorphisms with asthma. Furthermore, literatures about the genetic associations of STAT2, TLR4, and CD40 upon asthma are inconsistent. In this survey, our genetic targets were all import roles amongst the complex pathogenesis of asthma, including the cytokine signals (STAT), viral defense (TLR) and immune response (CD40). We aimed to evaluate whether STAT2, TLR4 and CD40 gene polymorphisms are attractive markers for predicting the susceptibility of asthma. We also performed linkage and association analyses in these candidate regions. To the best of our knowledge, this is the first survey in this field.

Patient and methods

All individuals were divided into two groups: (1) asthma (classification of asthma) (n=117); (2) normal controls (n=60). Taiwanese children with diagnosis of asthma were included. Asthmas was diagnosed as standard criteria, as previous describes [8]. The controls were consisted with health children. All individuals accepted the peripheral blood sampling for genotype analyses. The clinical data about the FEV1 or FEV1/FVC for the asthma individuals were also collected. The experiment was approved by Ethical Committee and Institutional Review Board of China Medical University Hospital.

The genomic DNA was prepared from peripheral blood leukocytes by use of a genomic DNA isolation kit (Blossom, Taipei, Taiwan). A total of 50 ng genomic DNA was mixed with 20 pmol of each polymerase chain reaction (PCR) primer in a total volume of 25 μl containing 10 mM Tris-HCL pH 8.3, 50 mM potassium chloride, 2.0 mM magnesium chloride, 0.2 mM each deoxyribonucleotide triphosphate, and 1 U DNA polymerase (Amplitag; Perkin-Elmer, Foster City, CA, USA). The PCR primer sequences and condition of each primer were listed in Table 1. The PCR amplification was performed in a programmable thermal cycler GenAmp PCR system 2400 (Perkin Elmer Applied Biosystems, Foster City, CA, USA).

After PCR amplification, the STAT2, TLR4 and CD40 gene polymorphisms were analyzed by restriction digestion with restriction enzymes (New England Biolabs, Inc, Beverly, MA). The restriction enzymes used for each DNA polymorphisms were listed in Table 1. Electrophoresis of the PCR product was performed on a 3 % agarose gel and stained with ethidium bromide to visualize the amplified DNA bands. The individual PCR conditions, following electrophoresis and base pairs for their wild and SNP types were listed in Table 1.

Table 1. The primer sequences, PCR conditions and restriction enzymes used in detecting STAT2, TLR4, and CD40 polymorphisms.

Gene (rs number)	Primer pairs	Alleles	Restriction Enzyme	Genotype: length of DNA fragments (bp)		Anneling Temperature (℃)
STAT2 (rs2066807)-F	5'-CTCGGAAGGTGGCTATTGTC-3'	C/G	Tth111I	CC:366	GG:244+122	TOUCH DOWN (51-60)
STAT2 (rs2066807)-R	5'-AAAGGAGAGGCTGTGGGAAT-3'					
TLR4(rs10983755)-F	5'-TCCACCTTGGATGACTATGT-3'	A/G	HpyCH4IV	AA:304	GG:243+61	58
TLR4(rs10983755)-R	5'-TATGCATGCTAAGTCCTAGA-3'					
TLR4(rs1927914)-F	5'- ACGTCTAGTCTAGAGCATCA -3'	C/T	NsiI	TT:270	CC:221+49	58
TLR4(rs1927914)-R	5'- ATTGGAAGTGCTTGGAGGAT -3'					
CD40 (rs1883832)-F	5'-TACACAGCAAGATGCGTCC CT-3'	C/T	NcoI	TT:291	CC:229+62	58
CD40 (rs1883832)-R	5'-AACAACTCACAGCGGTCAGCAA-3'					
CD40 (rs3765459)-F	5'-ATGCTCCTTCCATCCAGA -3'	A/G	HpyCH4III	GG:421	AA:263+158	58
CD40 (rs3765459)-R	5'-TCGTCGGGAAAATTGATCTC CT -3'					
CD40 (rs4810485)-F	5'-TTAGGAGACCAGAGTTCT-3'	G/T	MspI	TT:259+102	GG:148+111+102	58
CD40 (rs4810485)-R	5'-AAAGCTGTGGGACCAAAGCA-3'					

*F and R indicate forward and reverse primers

Genotypes and allelic frequencies for STAT2, TLR4 and CD40 gene polymorphisms in both groups were compared. Correlations of these gene polymorphisms and asthma were evaluated. Allelic frequencies are expressed as a percentage of the total number of alleles. The associations of different genotypes with FEV1 or FEV1/FVC for the asthma individuals were also estimated. The SAS package (Version 8.1, SAS Institute Inc., Cary, North Carolina, USA) with χ^2 and Fisher's extract tests were utilized for statistical analyses. A p-value of <0.05 was considered statistically significant.

Table 2. Genotypes and allelic frequencies for STAT2 gene polymorphism in individuals with and without asthma

STAT2 rs2066807	Asthma (n=117)	Control (n=60)	p*	OR	95% CI for OR
Genotype					
CC	0	0	0.03	7.38	0.94 57.80
CG	13 (11.1)	1 (1.7)			
GG	104 (88.9)	59 (98.3)			
Allele					
C	13 (5.6)	1 (0.8)	0.03	7.00	0.90 54.17
G	221 (94.4)	119 (99.2)			

* Fisher's extract tests

Results

The average age of onset and recruitment in the asthma group were 7.2±2.4 and 9.6±3.5 years, respectively. Genotype proportions of different gene polymorphisms of STAT2 in both groups were significantly different (Table 2, 3). Distributions of STAT2*C homozygote/CG heterozygote/G homozygote and C/G allele in both groups are: (1) 0/11.1/88.9 % and 5.6/94.4%; (2) 0/1.7/98.3% and 0.8/99.2% (p<0.05, Table 2). There is no individuals with STAT2*C homozygote.

STAT2*C related genotypes and allele are associated with higher susceptibility for asthma. In contrast, TLR4 polymorphisms are not associated with asthma development. Proportions of TLR4*rs 10983755 A homozygote/AG heterozygote/G homozygote in both groups are: (1) 35.1/8.5/56.4%; (2) 35/8.3/56.7% (non-difference, Table 3). Proportions of TLR4*rs1927914 C homozygote/CT heterozygote/TT homozygote in both groups are: (1) 9.4/56.4/34.2%; (2) 16.7/48.3/35% (non-difference, Table 3).

Table 3. Genotypes and allelic frequencies for TLR4 gene polymorphism in individuals with and without asthma.

	Asthma (n=117)	Control (n=60)	*p**	OR	95% CI for OR
TLR4 rs10983755					
Genotype					
AA	41 (35.1)	21 (35)	1.00	1.01	0.30 3.32
AG	10 (8.5)	5 (8.3)		1.03	0.33 3.26
GG	66 (56.4)	34 (56.7)			
Allele					
A	92 (39.3)	47 (39.2)	0.98	1.01	0.64 1.58
G	142 (60.7)	73 (60.8)			
TLR4 rs1927914					
Genotype					
CC	11 (9.4)	10 (16.7)	0.32	0.58	0.22 1.51
CT	66 (56.4)	29 (48.3)		1.19	0.60 2.37
TT	40 (34.2)	21 (35)			
Allele					
C	88 (37.6)	49 (40.8)	0.56	0.87	0.56 1.37
T	146 (62.4)	71 (59.2)			

*χ^2 test

Furthermore, three genetic variations for CD40 are not associated with asthma susceptibilities. Proportions of CD40*rs1883832 C homozygote/CT heterozygote/T homozygote in both groups are: (1) 29.9/53/17.1%; (2) 36.7/41.7/21.6% (non-difference, Table 4). Proportions of CD40*rs3765459 A homozygote/AG heterozygote/G homozygote in both groups are: (1) 6.8/47.9/45.3; (2) 1.6/46.7/ 51.7% (non-difference, Table 4). Proportions of CD40*rs4810485 TT homozygote/GT heterozygote/GG homozygote in both groups are: (1) 18.8/62.4/18.8%; (2) 15/51.7/33.3% (non-difference, Table 4). Concerning the association between the association of different halotypes for TLR4 and CD40 gene polymorphisms as well as their additional effects upon asthma risks, the statistical analyses revealed their non-association and absence of additional effect upon the asthma susceptibilities (Table 5, 6). Concerning the associations between STAT2 genotype and lung functions, we also observed the non-association between different genetic variation and FEV1 or FEV1/FVC values for the asthma individual (Table 7).

Table 4. Genotypes and allelic frequencies for CD40 gene polymorphism in individuals with and without asthma

	Asthma (n=117)	Control (n=60)	*p**	OR	95% CI for OR
CD40 rs1883832					
Genotype					
CC	35 (29.9)	22 (36.7)	0.36	1.034	0.510 2.097
CT	62 (53)	25 (41.7)		1.612	0.697 3.729
TT	20 (17.1)	13 (21.6)			
Allele					
C	132 (56.4)	69 (57.5)	0.84	0.957	0.613 1.492
T	102 (43.6)	51 (42.5)			
CD40 rs3765459					
Genotype					
AA	8 (6.8)	1 (1.6)	0.25	4.679	0.557 39.289
AG	56 (47.9)	28 (46.7)		1.170	0.620 39.289
GG	53 (45.3)	31 (51.7)			
Allele					
A	72 (30.8)	30 (25)	0.24	1.333	0.810 2.193
G	162 (69.2)	90 (75)			
CD40 rs4810485					
Genotype					
TT	22 (18.8)	9 (15)	0.10	2.222	0.920 5.369
GT	73 (62.4)	31 (51.7)		2.141	1.024 4.474
GG	22 (18.8)	20 (33.3)			
Allele					
T	117 (50)	49 (40.8)	0.10	1.449	0.928 2.262
G	117 (50)	71 (59.2)			

*χ^2 test

Table 5. Haplotype analysis for TLR4 gene polymorphisms

Haplotype	rs10983755	rs1927914	Asthma patients	Control	p	Odds ratio(95% CI)
Ht 1	G	T	0.462	0.442	0.721	1.08 (0.70-1.69)
Ht 2	A	C	0.225	0.242	0.719	0.91 (0.54-1.53)
Ht 3	A	T	0.162	0.167	0.904	0.96 (0.53-1.74)
Ht 4	G	C	0.151	0.15	0.980	1.01 (0.54-1.87)

Table 6. Haplotype analyses for CD40 gene polymorphisms

Haplotype	rs1883832	rs4810485	rs3765459	Asthma patients	Control	p	Odds ratio(95% CI)
Ht 5	T	T	G	0.402	0.389	0.813	1.06 (0.67-1.66)
Ht 6	C	G	A	0.263	0.225	0.435	1.23 (0.73-2.06)
Ht 7	C	G	G	0.208	0.335	0.009	0.52 (0.32-0.85)
Ht 8	C	T	G	0.048	0.025	0.297	1.97 (0.54-7.17)
Ht 9	C	T	A	0.041	0.012	0.137	3.52 (0.60-20.58)
Ht 10	T	G	G	0.032	0.013	0.284	2.51 (0.44-14.29)

Table 7. Association between STAT2 genotypes with FEV1 or FEV1/FVC.

STAT2 rs2066807	Asthma	FEV1 (%)	FEV1/FVC (%)	*p**
CC	0	0	0	NS
CG	13 (11.1)	70±12	68±13	
GG	104 (88.9)	73±15	72±18	

*: The differences were determined by univariated analysis of variances.

Discussion

Bronchial asthma is a chronic airway disorder characterized by reversible bronchial hyper-responsiveness and airway inflammation. Asthma is a multifactorial disease influenced by genetic and environmental factors. While environmental factors are critical for asthma development, genetic factors also play a major role in its clinical expressions [9]. Oxidative stress is a key component of inflammation. Inflammation is a key mechanism in asthma. Variation in genes encoding inflammatory responses might influence asthma risk through interaction with chronic inflammation and pro-inflammatory environmental risk factors, such as sedentary conditions, lifestyle, and air pollutions. However, the mechanism for asthma is complex. The precise physiological stimulus mediating asthma presentation remains obscure.

Growing evidences suggest that asthma is a multi-step process of genetic alterations. Some possible factors have been implicated with asthma, including cytokines, signal ligants, and defense factors. Genetic surveys for asthma might provide insight into related pathophysiology and mechanisms. During past decades, several loci and genes have been found to be associated with the disorder [10]. Numerous genetic factors might interfere with the inflammatory capacity of leukocytes, thus altering whole body allergy and asthma events. Some patients with different clinical phenotypes might display variable susceptibilities toward asthma.

STAT is associated with endothelial expression, transcription and regulations of cytokines, including interleukin, nitric oxide synthesis, p21 and interferon [11]. STAT function has been implicated in the transduction of signals for growth, reproduction, viral defense, and immune regulation [12]. STAT cascade are required for cytokines, growth factors, G-proteins and hormones (growth hormone and prolactin) [13]. Expression of some cytokines could be regulated by STAT proteins [14]. Transcription factors of STAT family are required for cellular responses to multiple signaling molecules [15]. After ligand binding-induced activation, STAT proteins are phosphorylated and translocated to the nucleus [16]. Then STAT binds to nuclear DNA elements in the promoters of specific genes, which further alter the transcriptional activity of these loci.

STAT gene is a positional candidate located on chromosome 2 [17]. STAT gene may be associated with predisposition to allergic diseases [18]. STAT gene represents one of the most promising candidate genes for asthma [19]. Numerous disorders were associated with STAT expressions, including asthma [3,20]. Leung *et al*. demonstrated the association between STAT6* C1570T genetic variation and lung function changes in asthma individuals [3]. Litonjua *et al*. indicated the STAT3 genetic polymorphism might participate in inflammatory pathways that have an impact on level of lung function [20]. Li *et al*. suggested that STAT-4 T90089C but not STAT-6 G2964A polymorphisms might be the genetic factors for the risk of asthma in Chinese population [21]. STAT gene might be involved in the development of eosinophilia and changes in total IgE levels in asthma individuals [19,22].

TLR, a key element in activating inflammatory cascade, plays a critical mediator of the immune response to pathogens. TLRs are highly conserved trans-membrane proteins that play an important role in the detection and recognition of microbial pathogens. Alterations of TLR signaling molecules might be associated with clinical presentation and susceptibility to infectious diseases such as asthma. TLRs play important roles in the signaling of many pathogen-related molecules and endogenous proteins associated with immune activation [23]. TLR signaling could induce the production of inflammatory cytokines and proteins in antigen presenting cells. Since

leukocyte adhesion is a critical event in airway inflammation and asthma, it is logical to suspect TLR might be involved in asthma pathogenesis. TLR pathway plays an important role in mediating whole body inflammation, which has been implicated in the development of chronic disease.

CD40 ligand is a transmembrane glycoprotein structurally related to tumour necrosis factor-alpha. CD40 ligation has been shown to promote antigen-presenting functions of immune cells, which express CD40 receptor. Some disorders have been demonstrated to be associated with CD40, including Graves' disease (GD) [24], atherosclerosis [25], multiple sclerosis [26]. CD40 gene polymorphism (C-1T)*C/C genotype has been reported to be associated with Graves' disease (GD) [24]. The interruption of CD40 signaling might produces a more fibrous and stable atherosclerotic lesion [25]. Carriers of T allele showed a trend for a lower stimulatory index compared with individuals with C homozygote, which might further interfere with the illness course of multiple sclerosis [26]. The CD40 protein might influence the production and function of immunoglobulin E [6].

Single nucleotide polymorphism (SNP) results from a base substitution mutation. Some cytokine polymorphisms have been reported to be protective or susceptible associated with asthma [27]. SNPs in protein-coding regions might result in a missense mutation (synonymous), with a change of amino acids or a nonsense mutation (non-synonymous) occurring in a termination codon. In addition, SNPs in promoter regions could result in reduced or increased gene expression, whereas SNPs in introns could result in defective splicing or a change in transcription rate if a regulatory element is mutated. SNPs occur on average every 1.9 kb in the genome where 1.42 million SNPs have been mapped with over 60,000 being represented within exons and untranslated regions [28]. In this study, we firstly observed some association existed between asthma susceptibility and STAT2 genetic variations. It suggested that STAT gene might be susceptibility genes for asthma.

Based on this association and linkage surveys, it is logical to speculate that STAT2*C related genotype alterations could be primarily responsible for the aberrant immune response that characterizes asthma. STAT2*C related genotypes and allele variants might directly or indirectly influence the mRNA translations for innate immune events. The STAT2 genetic variation might result in the synonymous coding change. Therefore, it is plausible to suspect the intervention, modification, determination or involvement of these STAT2 genetic variations upon the expression or stability of STAT2 as well as the following pathogenesis of asthma.

In this survey, we observed the non-association between STAT2 genotypes with FEV1 or FEV1/FVC, which suggested its roles upon asthma susceptibility rather than severities. Furthermore, there are also predictive values about its effects upon protein sequence. It suggested some STAT2 polymorphisms, might be associated with asthma risk as well as playing potential candidate genetic markers in predicting the susceptibility of asthma. There is biological plausibility for an association between the STAT2 polymorphisms in the exon or promoter regions and asthma risk. Our study should permit a more precise evaluation of the risks associated with individual susceptibility genes and a better insight into asthma pathogenesis. These polymorphisms might have potential influences upon the expression of these repair proteins. However, the real roles and relationships of these genetic traits upon asthma remain complex to be clarified, especially concerning the effects of smoking or life styles additions.

In this survey, we also observed the non-correlations of asthma with the TLR4 and CD40 polymorphisms. This intervening sequence located on mRNA-untranslated region might not influence the amino acid coding, mRNA production, genetic expression and illness susceptibilities. These findings suggested some genetic variations within the TLR4 and CD40 might not be associated with the genetic presentation such as transcription and translations as well as asthma phenotypes and susceptibilities. In addition, the additional effects among TLR4 and CD40 halotypes might not be associated with the susceptibility and contribution for asthma. To the best of our knowledge, this is among the first few study to address the issue of an interaction between genetic variations of STAT2, TLR4 and CD40 and asthma risk.

Taken together, STAT2*C genotype and allele might be correlated with asthma development and pathogenesis. In contrast, TLR4 and CD40 polymorphisms as well as the additional effects of their halotypes were not associated with the different susceptibility and contribution for asthma. These findings highlighted the values and potentials of the STAT2-realted genes upon the future surveys of asthma. STAT2 rs2066807 polymorphisms might become potential markers for the prediction of asthma susceptibility. It also provides a valuable insight into the pathogenesis of asthma. Additional *in-vitro* or *in-vivo* researches are requested, including functional studies correlating genotype and phenotype for specific STAT2 alleles within endothelium tissues for

airway. After the clarification of these issues, some STAT2 genetic variations might become useful markers to predict the future development of novel therapies for asthma as well as the modulating or interfering factors of related pathogeneses.

Conflict of Interest

The authors have declared that no conflict of interest exists.

References

1. Moorman JE, Rudd RA, Johnson CA, King M, Minor P, Bailey C, Scalia MR, Akinbami LJ. Centers for Disease Control and Prevention (CDC): National surveillance for asthma--United States, 1980-2004. MMWR Surveill Summ. 2007;56:1-54.
2. Mahdaviani SA, Rezaei N, Moradi B, Dorkhosh S, Amirzargar AA, Movahedi M. Proinflammatory Cytokine Gene Polymorphisms among Iranian Patients with Asthma. J Clin Immunol. 2008 [Epub ahead of print]
3. Leung TF, Chan IH, Wong GW, Li CY, Tang NL, Yung E, Lam CW. Association between candidate genes and lung function growth in Chinese asthmatic children. Clin Exp Allergy. 2007;37:1480-6.
4. Pinto LA, Steudemann L, Depner M, Klopp N, Illig T, Weiland SK, von Mutius E, Kabesch M. STAT1 gene variations, IgE regulation and atopy. Allergy. 2007;62:1456-1461.
5. Schumann RR, Tapping RI. Genomic variants of TLR1--it takes (TLR-)two to tango. Eur J Immunol. 2007;37:2059-62.
6. Park JH, Chang HS, Park CS, Jang AS, Park BL, Rhim TY, Uh ST, Kim YH, Chung IY, Shin HD. Association analysis of CD40 polymorphisms with asthma and the level of serum total IgE. Am J Respir Crit Care Med. 2007;175:775-82.
7. Burgess JK, Blake AE, Boustany S, Johnson PR, Armour CL, Black JL, Hunt NH, Hughes JM. CD40 and OX40 ligand are increased on stimulated asthmatic airway smooth muscle. J Allergy Clin Immunol. 2005;115:302-8.
8. National Heart Lung and Blood Institute. Global Initiative for Asthma - National Institutes of Health pub no 95-3659. US: NIH. 1995.
9. Movahedi M, Mahdaviani SA, Rezaei N, Moradi B, Dorkhosh S, Amirzargar AA. IL-10, TGF-beta, IL-2, IL-12, and IFN-gamma cytokine gene polymorphisms in asthma. J Asthma. 2008;45:790-4.
10. Balaci L, Spada MC, Olla N, Sole G, Loddo L, Anedda F, Naitza S, Zuncheddu MA, Maschio A, Altea D, Uda M, Pilia S, Sanna S, Masala M, Crisponi L, Fattori M, Devoto M, Doratiotto S, Rassu S, Mereu S, Giua E, Cadeddu NG, Atzeni R, Pelosi U, Corrias A, Perra R, Torrazza PL, Pirina P, Ginesu F, Marcias S, Schintu MG, Del Giacco GS, Manconi PE, Malerba G, Bisognin A, Trabetti E, Boner A, Pescollderungg L, Pignatti PF, Schlessinger D, Cao A, Pilia G. IRAK-M is involved in the pathogenesis of early-onset persistent asthma. Am J Hum Genet. 2007;80:1103-14.
11. Melchers I, Blaschke S, Hecker M, Cattaruzza M. The -786C/T single-nucleotide polymorphism in the promoter of the gene for endothelial nitric oxide synthase: insensitivity to physiologic stimuli as a risk factor for rheumatoid arthritis. Arthritis Rheum. 2006;54:3144-51.
12. Oates AC, Wollberg P, Pratt SJ, Paw BH, Johnson SL, Ho RK, Postlethwait JH, Zon LI, Wilks AF. Zebrafish stat3 is expressed in restricted tissues during embryogenesis and stat1 rescues cytokine signaling in a STAT1-deficient human cell line. Dev Dyn. 1999;215:352-70.
13. Buslei R, Kreutzer J, Hofmann B, Schmidt V, Siebzehnrübl F, Hahnen E, Eyupoglu IY, Fahlbusch R, Blümcke I. Abundant hypermethylation of SOCS-1 in clinically silent pituitary adenomas. Acta Neuropathol (Berl). 2006;111:264-71.
14. Chen B, He L, Savell VH, Jenkins JJ, Parham DM. Inhibition of the interferon-gamma/signal transducers and activators of transcription (STAT) pathway by hypermethylation at a STAT-binding site in the p21WAF1 promoter region. Cancer Res. 2000;60:3290-8.
15. Ambrosio R, Fimiani G, Monfregola J, Sanzari E, De Felice N, Salerno MC, Pignata C, D'Urso M, Ursini MV. The structure of human STAT5A and B genes reveals two regions of nearly identical sequence and an alternative tissue specific STAT5B promoter. Gene. 2002;285:311-8.
16. Oates AC, Wollberg P, Pratt SJ, Paw BH, Johnson SL, Ho RK, Postlethwait JH, Zon LI, Wilks AF. Zebrafish stat3 is expressed in restricted tissues during embryogenesis and stat1 rescues cytokine signaling in a STAT1-deficient human cell line. Dev Dyn. 1999;215:352-70.
17. Diosdado B, Monsuur AJ, Mearin ML, Mulder C, Wijmenga C. The downstream modulator of interferon-gamma, STAT1 is not genetically associated to the Dutch coeliac disease population. Eur J Hum Genet. 2006;14:1120-4.
18. Tamura K, Arakawa H, Suzuki M, Kobayashi Y, Mochizuki H, Kato M, Tokuyama K, Morikawa A. Novel dinucleotide repeat polymorphism in the first exon of the STAT-6 gene is associated with allergic diseases. Clin Exp Allergy. 2001;31:1509-14.
19. Duetsch G, Illig T, Loesgen S, Rohde K, Klopp N, Herbon N, Gohlke H, Altmueller J, Wjst M. STAT6 as an asthma candidate gene: polymorphism-screening, association and haplotype analysis in a Caucasian sib-pair study. Hum Mol Genet. 2002;11:613-21.
20. Litonjua AA, Tantisira KG, Lake S, Lazarus R, Richter BG, Gabriel S, Silverman ES, Weiss ST. Polymorphisms in signal transducer and activator of transcription 3 and lung function in asthma. Respir Res. 2005;6:52.
21. Li Y, Wu B, Xiong H, Zhu C, Zhang L. Polymorphisms of STAT-6, STAT-4 and IFN-gamma genes and the risk of asthma in Chinese population. Respir Med. 2007;101:1977-81.
22. Weidinger S, Klopp N, Wagenpfeil S, Rümmler L, Schedel M, Kabesch M, Schäfer T, Darsow U, Jakob T, Behrendt H, Wichmann HE, Ring J, Illig T. Association of a STAT 6 haplotype with elevated serum IgE levels in a population based cohort of white adults. J Med Genet. 2004;41:658-63.
23. Tahara T, Arisawa T, Wang F, Shibata T, Nakamura M, Sakata M, Hirata I, Nakano H. Toll-like receptor 2 -196 to 174del polymorphism influences the susceptibility of Japanese people to gastric cancer. Cancer Sci. 2007;98:1790-4.
24. Kurylowicz A, Kula D, Ploski R, Skorka A, Jurecka-Lubieniecka B, Zebracka J, Steinhof-Radwanska K, Hasse-Lazar K, Hiromatsu Y, Jarzab B, Bednarczuk T. Association of CD40 gene polymorphism (C-1T) with susceptibility and phenotype of Graves' disease. Thyroid. 2005;15:1119-24.
25. Burdon KP, Langefeld CD, Beck SR, Wagenknecht LE, Carr JJ, Rich SS, Freedman BI, Herrington D, Bowden DW. Variants of the CD40 gene but not of the CD40L gene are associated with coronary artery calcification in the Diabetes Heart Study (DHS). Am Heart J. 2006;151:706-11.
26. Buck D, Kroner A, Rieckmann P, Mäurer M, Wiendl H. Analysis of the C/T(-1) single nucleotide polymorphism in the CD40 gene in multiple sclerosis. Tissue Antigens. 2006;68:335-8.
27. Trajkov D, Stojkovikj JM, Arsov T, Petlichkovski A, Strezova A, Mladenovska OE, Sandevska E, Gogusev J, Spiroski M. Association of cytokine gene polymorphisms with bronchial asthma in macedonians. Iran J Allergy Asthma Immunol. 2008;7:143-56.

28. Marth G, Yeh R, Minton M, Donaldson R, Li Q, Duan S, Davenport R, Miller RD, Kwok PY. Single-nucleotide polymorphisms in the public domain: how useful are they? Nat Genet. 2001;27:371-2.