ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
March 2013; 12(1): 29-36.

MCP-1, CCR2 and CCR5 Polymorphisms in Tunisian Patients with Atopic Asthma

Tarak Dhaouadi[1], Imen Sfar[1], Hajer Aounallah-Skhiri[2], Saloua Jendoubi-Ayed[1], Hend Bouacha[3], Taieb Ben Abdallah[1], and Yousr Gorgi[1]

[1]Laboratory of Research in Immunology of Renal Transplantation and Immunopathology, Charles Nicolle Hospital, Tunis El Manar University, Tunis, Tunisia
[2]National Institute of Public Health, Tunis, Tunisia
[3]Pneumonology Department, Charles Nicolle Hospital, Tunis, Tunisia

Received: 3 November 2011; Received in revised form: 8 May 2012; Accepted: 8 August 2012

ABSTRACT

Chemokines and their receptors play an important role in the late inflammatory stage of asthma. In this study, we aimed to investigate polymorphisms of MCP-1 (CCL2), CCR2 and CCR5 which can affect qualitatively and/or quantitatively their production and thus influence both susceptibility and severity of asthma and its clinical and biological features.

MCP-1 (A/G -2518), CCR2 (+/64I), CCR5 (G/A -59029) and CCR5 (Δ32) polymorphisms were evaluated by PCR in 107 Tunisian patients with asthma and 169 healthy controls.

No significant association was found between the four investigated polymorphisms and asthma. Nevertheless the haplotype MCP1*AG/CCR2*+/+ was significantly l ess frequent in patients (20.5%) compared to controls (32.5%) (p=0.03; OR=0.54; 95% CI: 0.29-0.98). Whereas no difference was observed in CCR2/CCR5 haplotypes between patients and controls. Analysis of polymorphisms with clinical and biological features showed that the concomitant presence of MCP-1*G/CCR2*64I alleles was less frequent in severe forms (4.34%) compared to moderate disease (12%) but the difference was not significant (p=0.27). No association was observed between the four polymorphisms and the presence of atopic rhinitis or atopic conjunctivitis and an elevated rate of serum IgE over 200 IU/ml.

Additional effects of MCP-1 and its receptor CCR2 polymorphisms seem to be involved in disease susceptibility to asthma in Tunisian patients; nevertheless they could be protective against its severe forms.

Keywords: Asthma; Atopy; Chemokines; MCP-1; Polymorphism

Corresponding Author: Yousr GORGI, MD;
Laboratory of Research in Immunology of Renal Transplantation and Immunopathology (LR03SP01), Charles Nicolle Hospital, Tunis El Manar University, Bd 9 Avril, 1006 Bab Saadoun, Tunis, Tunisia.
Tel: (+216 71) 578 055, Fax: (+216 71) 561 156, E-mail: gorgi.yousr@gmail.com

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

INTRODUCTION

Asthma is a pulmonary disease characterized by an increased bronchial responsiveness to a variety of stimuli. Since previous studies had suggested that asthma is a multi-factorial disease, influenced by genetic and environmental factors about 30 years ago,[1] a bundle of genome-wide screens for asthma were performed.[2-6] About 6 regions (2q, 5q, 6q, 11q, 12q and 13q) were found to be highly linked with asthma and extensively replicated in many studies.[2-6] Twenty-one genes, such as CTLA-4, CCR5, IL4, IL13, TNF, HLA-G, FcεR1 and RANTES, were identified through association studies and reproduced in subsequent investigations.[7]

Chemotactic cytokines or chemokines are small signaling proteins that are deeply involved in acute and chronic inflammatory processes by attracting and/or stimulating specific subsets of leukocytes.[8] Moreover, production of a number of chemokines have been identified to be related to the severity of asthmatic inflammation and reactive airway responses.[9,10]

Monocyte chemoattractant protein 1 (MCP-1) or CCL2 in the newest nomenclature, might play a significant role in allergic responses because of its ability to induce mast cell activation and leukotriene C4 release into the airways through its receptor CCR2, which directly induces airway hyper-responsiveness.[11] In addition, neutralization of MCP-1 drastically reduces bronchial hyper-reactivity, lymphocyte-derived inflammatory mediators, and T-cell and eosinophil recruitment into the lung.[12] A single nucleotide polymorphism (SNP) in the promoter region of MCP-1 (A/G -2518) has been described and found to increase the level of MCP-1 expression in response to inflammatory stimuli.[13]

Similarly, it was reported that a SNP (G to A) at position 190 of the gene encoding the first CCR2 transmembrane region of the protein, results in a change from valine to isoleucine at codon 64 (64I) of the polypeptide chain. Whereas levels of mRNA seem to be very similar in peripheral blood mononuclear cells in both mutated and homozygous wild-type allele subjects, cell surface expression is considerably decreased in case of mutated allele and might be due to post-transcriptional regulatory mechanisms that influence the expression of CCR2.[14]

RANTES is one of the most extensively studied chemokines in allergic diseases and is likely to be important in airway inflammation as its blockade by monoclonal antibodies inhibits airway inflammation in a murine model of allergic airway disease.[15] Besides, eosinophil chemotactic activity that appears in the bronchoalveolar lavage fluid of patients with asthma after allergen challenge was found to be due to RANTES.[16] CCR5 is the receptor of RANTES and MIP-1. CCR5 polymorphisms were widely investigated in HIV infection as it represents the major receptor allowing the virus to penetrate into cells.[17] A deletion of 32 base-pair (bp) in the open reading frame (Δ32) of CCR5 gene encodes a non-functional protein.[18] Only few studies had investigated this Δ32 mutation in patients with asthma and results were quite conflicting. Another SNP in the CCR5 promoter was described as a G to A substitution at position -59029 resulting in a decrease of its activity at 45%.[17]

In this study, polymorphisms of MCP-1 (A/G -2518), CCR2 (64I) and CCR5 (Δ32 and G/A -59029) genes were investigated in order to detect a possible association of allelic variants with susceptibility to asthma and its clinical and biological features.

MATERIALS AND METHODS

Patients and Control Subjects

This retrospective study involved 107 patients with atopic asthma (Table 1). All patients were visiting the pneumonology department of the Charles Nicolle University Hospital in Tunis (Tunisia) and were diagnosed as asthmatic patients. The disease severity was estimated according to the Global Initiative for Asthma (GINA) 2002 revised criteria.[19] Data obtained from each patient included age at diagnosis, gender, clinical and biological features. Patients with a severe persistent asthma and nocturnal symptoms were considered as having a severe disease.

As a control group, we studied 169 healthy voluntary blood donors from the same geographic origin and matched for age, gender and ethnicity. None of the healthy subjects had any evidence of allergic or asthmatic disease.

All patients and controls gave informed consent to participate in the study, and the local Ethics committee of Charles Nicolle Hospital approved the study.

Methods

Genomic DNA was extracted from the cells of the peripheral blood using salting-out procedure.[20]

Table 1. Clinical and biological features of patients

Sex Ratio; Female/male	76/36
Mean Age± SD; years	26.81 ± 11.67
Median evolution; months	78.64
Associated allergic manifestations (rhinitis and/or conjunctivitis); n (%)	86 (80.37%)
Family history of allergy; n (%)	28 (26.16%)
Family history of asthma; n (%)	11 (10.2%)
Disease forms; n (%)	
Severe	23 (21.49%)
Moderate	84 (78.5%)
IgE> 200 IU/ml; n (%)	66 (61.68%)

n: number

MCP-1 Promoter Genotyping (A/G -2518)

The identification of polymorphism was carried out using PCR, followed by a restriction fragment length polymorphism (RFLP) assay, using *PvuII* site, which is introduced by the presence of the G nucleotide. The PCR reaction was carried out in a 20 µl reaction mixture containing 100 ng/µl genomic DNA, 0.2 mMol dNTP, 1.5 mMol $MgCl_2$, 5 µMol of each forward and reverse primers, consigned in table 2 and 0.5 U Taq DNA polymerase (Promega, USA).

PCR was run for 40 cycles using the following temperature profile: denaturation at 94°C for 60sec, annealing at 55°C for 60 sec, extension at 72°C for 90 sec followed by a single final extension step at 72°C for 10 min. The PCR products result in DNA fragment of 930 bp. Four µl of these PCR products were digested with 5U of *PvuII* in 10x buffer and H_2O up to a final volume of 20 µl at 37°C for 2.5 hrs. The digested products were loaded into 1.5% agarose gels and stained by ethidium bromide. Samples showing only a 930 bp were assigned as A/A, samples showing two bands of 708 bp and 222 bp were considered G/G and samples showing three bands 930, 708 and 222 bp were typed A/G.

CCR2 (64I) Genotyping

For characterization of the CCR2 polymorphism, a sequence specific primer PCR was used (SSP-PCR) with the primers listed in Table 2. The PCR reaction was carried out in a final volume of 30 µl with 100ng/µl genomic DNA, 0.2 mMol dNTP, 2 mMol $MgCl_2$, 5 µMol of each primer (Table 2) and 0.25 U Taq DNA polymerase.

PCR was run for 5 cycles using a single initial denaturation at 94°C for 1 min, 5 cycles of denaturation at 96°C for 25 sec, annealing at 65°C for 50 sec, extension at 72°C for 45 sec, 21 cycles of denaturation at 96°C for 25 sec, annealing at 70°C for 45 sec, extension at 72°C for 45 sec, and 4 cycles of denaturation at 96°C for 25 sec, annealing at 55°C for 60 sec, extension at 72°C for 2 min.

Table 2. Sequences of used primers

MCP-1 A/G -2518	Primers
Forward primer	5'CCGAGATGTTCCCAGCCAG-3'
Reverse primer	5'-CTGCTTTGCTTGTGCCTCTT-3'
CCR2 (64I)	
Specific primer 1	CCR2 440 5'-GTGGGCAACATGCTGGTCA-3'
Specific primer 2	CCR2 442 5'-GTGGGCAACATGCTGGTCG-3'
Common primer	CCR2 441 5'-CCCAAAGACCCACTCATTTG-3'
CCR5 G/A -59029	
Forward primer	5'-CCCGTGAGCCCATAGTTAAAACTC-3'
Reverse primer	5'-TCACAGGGCTTTTCAACAGTAAGG-3'
CCR5 Δ32	
Forward primer	5'-TGTTTGCGTCTCTCCCAG-3'
Reverse primer	5'-CACAGCCCTGTGCCTCTT-3'

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

CCR5 Promoter (G/A -59029) Genotyping

CCR5-59029 genomic variants were detected by using RFLP-PCR. The presence of the G nucleotide at position -59029 of the CCR5 gene creates a recognition site for the *BspI286*I enzyme. The PCR reaction was carried out in a 20 µl reaction mixture containing 100 ng/µl genomic DNA, 0.175 mMol dNTP, 1.5 mMol $MgCl_2$, 10 µMol of each forward and reverse primers (table 2) and 0.5 U Taq DNA polymerase.

PCR was run for 35 cycles using the following temperature profile: a single initial denaturation at 94°C for 4 min, denaturation at 94°C for 30 sec, annealing at 55°C for 45 sec, extension at 72°C for 1 min, followed by a single final extension step at 72°C for 7 min. 5µl of the PCR products were digested with 3U of *BspI286*I in 10X buffer and H_2O up to a final volume of 20 µl at 37°C for 1 night. The resulting products were separated by gel-electrophoresis in 2% agarose gels. Samples showing only a 130 bp were assigned as G/G, samples showing only 258 bp band were considered A/A and heterozygotes showed both bands.

CCR5Δ32 Genotyping

CCR5Δ32 genotype was determined by sizing PCR amplicons that included the entire region of the deletion. The PCR reaction was carried out in a 20 µl reaction mixture containing 50 ng/µl genomic DNA, 0.175 mMol dNTP, 1.5 mMol $MgCl_2$, 5pMol of each forward and reverse primers (Table 2) and 0.5 U Taq DNA polymerase.

Thermocycling procedure consisted of initial denaturation at 94°C for 4 min, followed by 35 cycles of 94°C for 30 sec, 52°C for 45 sec, 72°C for 7 min. Amplicons were visualized in 2% agarose gels and revealed a 233 bp product for the wild type allele and 201 bp for the deletion product.

Statistical Methods

Univariate analysis was performed using chi-square test or fisher's exact test for small numbers (Epi-info Stat 6.04 program CDC, Atlanta). Probability (*p*) values were corrected for the number of tested alleles (pc). Values <0.05 were considered to be statistically significant. Frequencies of genotypes, alleles and phenotypes were analyzed by chi-square test. In order to evaluate the strength of associations, the odds ratios (OR) together with 95% confidence intervals (CI) were calculated. Logistic regression models were built according to age and gender to estimate adjusted ORs.

RESULTS

Epidemiological Characteristics

The study consisted of 71 females and 36 males with a female to male ratio of 2.1. Family history of allergy and asthma was positive in 28 and 11 patients, respectively. Eighty six patients had associated atopic rhinitis and/or conjunctivitis. Using GINA criteria, merely 23 patients had a severe disease. Biologically, IgE was over 200 IU/ml in 66 patients (Table 1).

Genetic Polymorphisms

All analyzed allele frequencies and genotype distributions were in Hardy-Weinberg equilibrium in both patients and controls. Results of polymorphism frequencies are summarized in table 3.

MCP-1 (A/G -2518)

There were no significant differences in allele and genotype frequencies between patients and controls. Allele G, though not significantly, was less frequent in patients with severe forms (0.196) compared to patients with moderate forms (0.238). There were no associations between any of alleles and genotypes and associated atopic manifestations (rhinitis and/or conjunctivitis) or an elevated IgE level over 200 IU/ml.

CCR2 (+/64I)

Allele and genotype distribution was quite similar in patients and controls. Furthermore, we observed no discrepancy in allele and genotype frequencies in patients with atopic manifestations or with IgE> 200 IU/ml. However, 64I allele was quite less frequent in patients with a severe asthma compared to the other remaining patients but the difference was not statistically significant.

CCR5 (Δ32) and (G/A -59029)

We found no significant differences in allele and genotype frequencies between patients and controls. Additionally, there were no associations between the wild-type allele and cases with associated atopic manifestations (rhinitis and/or conjunctivitis) or patients with an elevated IgE level over 200 IU/ml or severe asthma.

Table 3. MCP-1, CCR2 and CCR5 genotype and allele frequencies in patients and controls

Polymorphism	Controls (n=169)	Patients (n=107)	Persistent asthma (n=23)	Atopic association (n=83)	IgE>200 IU/ml (n=66)
MCP-1 (A/G -2518)					
Genotype					
AA	90 (53.2%)	68 (63.5%)	15 (65.21%)	57 (68.6%)	42 (63.3%)
AG	67 (39.6%)	29 (27.1%)	7 (30.4%)	18 (21.6%)	17 (25.7%)
GG	12 (7.1%)	10 (9.3%)	1 (4%)	8 (9.6%)	7 (10.6%)
Allele					
A	0.73	0.771	0.804	0.795	0.765
G	0.269	0.228	0.196	0.205	0.235
CCR2 (64I)					
Genotype					
+/+	124 (73.3%)	67 (62.6%)	17 (73.91%)	51 (61.4%)	38 (57.5%)
+/64I	41 (24.2%)	37 (34.5%)	5 (21.73%)	29 (34.9%)	26 (39.39%)
64I/64I	4 (2.3%)	3 (2.8%)	1 (4.34%)	3 (3.61%)	2 (3%)
Allele					
+	0.855	0.799	0.847	0.771	0.772
64I	0.145	0.200	0.153	0.229	0.228
CCR5 (Δ32)					
Genotype					
+/+	161 (95.2%)	100 (93.4%)	21 (91.3%)	78 (93.9%)	63 (95.4%)
+/Δ32	8 (4.7%)	7 (6.5%)	2 (8.6%)	5 (5.3%)	3 (4.5%)
Δ32/Δ32	0	0	-	-	-
Allele					
+	0.976	0.967	0.956	0.969	0.977
Δ32	0.023	0.032	0.043	0.030	0.022
CCR5 (G/A -59029)					
Genotype					
GG	47 (27.8%)	24 (22.4%)	6 (26%)	21 (25.3%)	18 (27.2%)
GA	88 (52%)	58 (54.2%)	10 (43.4%)	45 (54.2%)	33 (50%)
AA	34 (20.1%)	25 (23.36%)	7 (30.4%)	17 (20.4%)	15 (22.7%)
Allele					
G	0.538	0.495	0.478	0.524	0.522
A	0.461	0.504	0.521	0.476	0.578

Haplotype Distribution

Investigation of haplotype distribution between MCP-1 (A/G -2518) and its receptor CCR2 (64I) showed a significant decrease of the haplotype MCP-1*A/*G/CCR2*+/*+in patients (20.5%) compared to controls (32.5%) (p=0.03 OR=0.54 CI: 0.29-0.98) (Table 4). Inversely, the concomitant presence of MCP-1*G/CCR2*64I alleles was less frequent in severe forms (4.34%) than in patients with a moderate asthma (12%) but the difference was not statistically significant (p=0.27).

We found no differences in CCR2/CCR5 haplotype frequencies between patients and controls, and no associations with any of clinical and biological features.

After adjustment for age and gender, multivariate analysis did not reveal any statistical association for the allele and genotype frequencies studied.

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

Table 4. MCP-1/CCR2 Haplotype distribution

Haplotype MCP-1/CCR2	Controls (n=169)	Patients (n=107)	Persistent asthma (n=23)	Atopic association (n=83)	IgE> 200 IU/ml (n=66)
AA / +/+	61 (36%)	41 (38.3%)	10 (43.4%)	33 (39.75%)	23 (34.84%)
AA / +/64I	27 (15.9%)	24 (22.4%) NS	4 (17.39%)	21 (25.3%)	17 (25.75%)
AA / 64I/64I	2 (1.18%)	3 (2.8%)	1 (4.3%)	3 (3.6%)	2 (3%)
AG / +/+	55 (32,5%)	**22 (20.5%) *p*=0.03***	7 (30.4%)	15 (18%)	13 (19.69%)
AG / +/64I	10 (5,9%)	7 (6.5%)	0	3 (3.6%)	4 (6%)
AG / 64I/64I	2 (1,18%)	0	0	0	0
GG / +/+	8 (4,7%)	4 (3.7%)	0	3 (3.6%)	2 (3%)
GG / +/64I	4 (2.36%)	6 (5.6%)	1 (4.3%)	5 (6%)	5 (7.5%)
GG / 64I/64I	0	0	0	0	0

* *p* comparing MCP-1*AG / CCR2*+/+ haplotype between patients and controls

DISCUSSION

In Tunisian patients with atopic asthma, only few studies have focused on both chemokines and chemokine receptors and to our knowledge, this is the first study to publish data on a potential association of polymorphisms occurring in their genes with both disease susceptibility and severity.

MCP-1 was found to mediate airway hyper-reactivity in normal and allergic mice and directly induced mast cell degranulation through its receptor CCR2 *in vitro.*[11] Several human studies have demonstrated that MCP-1 is up-regulated during asthmatic responses.[21,22] The expression of MCP-1 in bronchial tissue from asthmatic subjects was found to be significantly increased in comparison with that in non-asthmatic subjects, >50% and <8%, respectively.[21] The -2518 A to G polymorphism in the gene promoter was found to affect the transcriptional activity of this region and was correlated with individual differences in MCP-1 production.[13] Szalai et al.[23] in a Caucasian population found that individual carriers for the high MCP-1 producer G allele were at increased risk for asthma and that the presence of allele G correlated with asthma severity and with an increased blood rate of eosinophils. Inversely, a study in Chinese children failed to show any association, neither with asthma nor with severity, and concluded that this SNP was not a risk factor for near-fatal asthma.[24] We also found no direct association of allele G with asthma, although there was a non-significant lower frequency of this allele in severe asthmatics. Moreover, another study investigated 64I polymorphism in CCR2 gene analysis and showed no difference between patients and controls and no correlation with severity, atopic associations and/or elevated titer of IgE. Contrary to these results, a study in an Indian population investigating CCR2 polymorphisms showed an association between these polymorphisms and total serum IgE levels.[25] In addition, in a Korean population, the presence of the 64l allele conferred significantly lower risk for the development of asthma [26]. Nevertheless, if our results failed to show a direct association of MCP-1 and CCR2 polymorphisms with asthma, we found that MCP-1*A/G/CCR2*+/+ haplotype is associated with lower risk for developing asthma. This result may be due to a weak role of each of polymorphisms taken alone, but additive contribution seem to be involved in disease-susceptibility to asthma. Even so, both MCP-1*G and CCR2*64l alleles were less frequent in patients with severe asthma and could be protective.

CCR5 is the receptor of RANTES (CCL5) which is of the most widely investigated chemotactic factor in atopic diseases and is likely to be important in airway inflammation. Above and beyond, eosinophil chemotactic activity that appears in the bronchoalveolar lavage fluid of patients with asthma after allergen challenge was found to be due to RANTES [16]. A deletion of 32 base-pair (bp) in the open reading frame (Δ32) of CCR5 gene encodes a non-functional protein.[18] We found no association of this polymorphism, neither with asthma susceptibility, nor with disease severity, nor with associated atopic manifestations nor with an elevated serum IgE over 200 IU/ml. Our results corroborate those found in two other

studies involving Iranian[27] and British[28] populations. Other studies reported that people with this mutation were at reduced risk for the developing asthma, and Δ32 allele could be considered to be protective.[29-31] In fact, this deletion results in reduced expression of CCR5 in cell membrane, leading to a resistance against RANTES secretion thus a lower risk for developing airway inflammation and eosinophil recruitment. We failed to find a protective role of this mutation in our study because of the lower frequency of this mutation in our general population, indeed if Δ32 allele prevalence is about 0.023 in our controls; it is approximately between 0.06 and 0.1 in Caucasians.

Till now, no other study has investigated the G to A at position -59029 of CCR5 gene in asthma. We found no association, neither with asthma nor with its clinical and biological features. This SNP was not shown to be a susceptibility factor to asthma in Tunisian population.

Additional effects of MCP-1 (A/G -2518) and its receptor CCR2 (64I) polymorphisms seem to be involved in disease-susceptibility to asthma in Tunisian patients; nevertheless they could be protective against its severe forms. Conversely, both CCR5 (Δ32 and G/A -59029) are likely to not interfere with asthma susceptibility and/or severity.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Tunisian Kidney Transplantation and Immunopathology Research Fund (LR03SP01).

REFERENCES

1. Marsh DG, Meyers DA, Bias WB. The epidemiology and genetics of atopic allergy. N Engl J Med 1981; 305(26):1551-9.
2. A genome-wide search for asthma susceptibility loci in ethnically diverse populations. The Collaborative Study on the Genetics of Asthma (CGSA). Nat Genet 1997; 15(4):389-92.
3. Koppelman GH, Stine OC, Xu J, Howard TD, Zheng SL, Kauffman HF, et al. Genome-wide search for atopy susceptibility genes in Dutch families with asthma. J Allergy Clin Immunol 2002; 109(3):498-506.
4. Ober C, Tsalenko A, Parry R, Cox NJ. A second-generation genomewide screen for asthma susceptibility alleles in a founder population. Am J Hum Genet 2000; 67 (5):1154-62.
5. Dizier MH, Besse-Schmittler C, Guilloud-Bataille M, Annesi-Maesano I, Boussaha M, Bousquet J, et al. Genome screen for asthma and related phenotypes in theFrench EGEA study. Am J Respir Crit Care Med 2000; 162(5):1812-8.
6. Daniels SE, Bhattacharrya S, James A, Leaves NI, Young A, Hill MR, et al. A genome-wide search for quantitative trait loci underlying asthma. Nature 1996; 383(6597):247-50.
7. Hoffjan S, Nicolae D, Ober C. Association studies for asthma and atopic diseases: a comprehensive review of the literature. Respir Res 2003; 4:14-28.
8. Baggiolini M. Chemokines and leukocyte traffic. Nature 1998; 392(6676):565-8.
9. Folkard SG, Westwick J, Millar AB. Production of interkeukin-8, RANTES and MCP-1 in intrinsic and extrinsic asthma. Eur Respir J 1997; 10(9):2097-104.
10. Lukacs NW, Oliveira SH, Hogaboam CM. Chemokines and asthma: redundancyn of function or a coordinated effort? J Clin Invest 1999; 104(8):995-99.
11. Campbell EM., Charo IF, Kunkel SL, Strieter RM, Boring L, Gosling J, et al. Monocyte chemoattractant protein-1 mediates cockroach allergeninducedbronchial hyperreactivity in normal but not CCR2-/- mice: the role of mast cells. J Immunol 1999; 163(4):2160-7.
12. Gonzalo JA, Lloyd CM, Wen D, Albar JP, Wells TN, Proudfoot A, et al. The coordinated action of CC chemokines in the lung orchestrates allergicinflammation and airway hyperresponsiveness. J Exp Med 1998; 188(1):157-67.
13. Rovin BH, Lu L, Saxena R. A novel polymorphism in the MCP-1 generegulatory region that influences MCP-1 expression. Biochem Bioph Res Commun 1999; 259(2):344-8.
14. Mariani R., Wong S, Mulder LC, Wilkinson DA, Reinhart AL, LaRosa G, et al. CCR2-64I polymorphism is not associated with altered CCR5 expression or coreceptor function. J Virol 1999; 73(3):2450-9.
15. Lukacs NW, Strieter RM, Warmington K, Lincoln P, Censue SW, Kunkel SL. Differential recruitment of leukocyte populations and alteration of airway hyperreactivity by C-C family chemokines in allergic airway inflammation. J Immunol 1997; 158(9):4398-404.
16. Teran LM, Noso N, Carrol M, Davies DE, Holgate S, Schroder JM. Eosinophil recruitment following allergen challenge is associated withthe release of the chemokine RANTES into asthmatic airways. J Immunol 1996; 157(4):1808-12.
17. McDermott DH, Zimmerman PA, Guignard F, Kleeberger CA, Leitman SF, Murphy PM. CCR5

promoter polymorphism and HIV-1 disease progression. Multicenter AIDS Cohort Study (MACS). Lancet 1998; 352(9131):866-70.
18. Huang Y, Paxton WA, Wolinsky SM, Neumann AU, Zhang L, He T, et al. The role of a mutant CCR5 allele in HIV-1 transmission and disease progression. Nat Med 1996; 2(11)**:**1240-43.
19. Global Initiative for Asthma. GINA Workshop Report, Global Strategy for Asthma Management and Prevention, April 2002. http://www.ginasthma.com/workshop.pdf.Updated November 2003.http://www.ginasthma.com/wrclean.pdf.
20. Miller SA, Dykes DD, Polesky HG. A sampling salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16(3):1215.
21. Sousa AR., Lane SJ, Nakhosteen JA, Yoshimura T, Lee TH, Poston RN. Increased expression of monocyte chemotactic protein-1 in bronchial tissue from asthmatic subjects. Am J Respir Cell Mol Biol 1994; 10(2):142-7.
22. Holgate ST, Bodey KS, Janezic A, Frew AJ, Kaplan AP, Teran LM. Release of RANTES, MIP-1a, and MCP-1 into asthmatic airways following endobronchial allergen challenge. Am J Respir Crit Care Med 1997; 156(5):1377-83.
23. Szalai C, Kozma GT, Nagy A, Bojszkó Á, Krikovszky D, Szabó T, et al. Polymorphism in the gene regulatory region of MCP-1 is associated with asthma susceptibility and severity. J Allergy Clin.Immunol 2001; 108(3):375-81.
24. Yao TC, Wu KC, Chung HT, Shaw CK, Kuo ML, Wu CJ, et al. MCP-1 gene regulatory region polymorphism in Chinese children with mild, moderate and near fatal asthma. Allergy 2004; 59(4):436-41.
25. Batra J, Ghosh B. Genetic contribution of chemokine receptor 2 (CCR2) polymorphisms towards increased serum total IgE levels in Indian asthmatics. Genomics 2009; 94(3):161-8.
26. Kim YK, Oh HB, Lee EY, Gho YS, Lee JE, Kim YY. Association between genetic variation of CC chemokine receptor-2 and atopic asthma. Allergy 2007; 62(2):208-9.
27. Abousaidi H, Vazirinejad R, Arababadi MK, Rafatpanah H, Pourfatollah AA, Derakhshan R, et al. Lack of association between chemokine receptor 5 (CCR5) δ32 mutation and pathogenesis of asthma in Iranian patients. South Med J 2011; 104(6):422-5.
28. Mitchell TJ, Walley AJ, Pease JE, Venables PJ, Wiltshire S, Williams TJ, et al. Delta 32 deletion of CCR5 gene and association with asthma or atopy. Lancet 2000; 356(9240):1491-2.
29. Srivastava P, Helms PJ, Stewart D, Main M, Russel G. Association of CCR5Δ32 with reduced risk of childhood but not adult asthma. Thorax 2003; 58(3):222-6.
30. McGinnis R, Child F, Clayton S, Davies S, Lenney W, Illig T, et al. Further support for the association of CCR5 allelic variants with asthma susceptibility. Eur J Immunogenet 2002; 29(6):525-8.
31. Hall IP, Wheatley A, Christie G, McDougall C, Hubbard R, Helms PJ. Association of CCR5Δ32 with reduced risk of asthma. Lancet 1999; 354(9186):1264-5.