Association Analysis of Monocyte Chemotactic Protein-3 (MCP3) Polymorphisms with Asthmatic Phenotypes

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The monocyte chemotactic protein-3 (MCP3), on chromosome 17q11.2-q12, is a secreted chemokine, which attracts macrophages during inflammation and metastasis. In an effort to discover additional polymorphism(s) in genes whose variant(s) have been implicated in asthma, we scrutinized the genetic polymorphisms in MCP3 to evaluate it as a potential candidate gene for asthma host genetic study. By direct DNA sequencing in twenty-four individuals, we identified four sequence variants within the 3 kb full genome including 1,000bp promoter region of MCP3; one in promoter region (-420T>C), three in intron (+136C>G, +563C>T, +984G>A) respectively. The frequencies of those four SNPs were 0.020 (-420T>C), 0.038 (+136C>G), 0.080 (+563C>T), 0.035 (+984G>A), respectively, in Korean population (n = 598). Haplotypes, their frequencies and linkage disequilibrium coefficients (|D'|) between SNP pairs were estimated. The associations with the risk of asthma, skin-test reactivity and total serum IgE levels were analyzed. Using statistical analyses for association of MCP3 polymorphisms with asthma development and asthma-related phenotypes, no significant signals were detected. In conclusion, we identified four genetic polymorphisms in the important MCP3 gene, but no significant associations of MCP3 variants with asthma phenotypes were detected. MCP3 variation/haplotype information identified in this study will provide valuable information for future association studies of other allergic diseases.

Keywords: Asthma, Allergic disease, MCP3, Single nucleotide polymorphism

Introduction

Asthma is a common and heterogeneous respiratory disease characterized by intermittent airway obstruction and respiratory symptoms that are caused by chronic airway inflammation. The development of asthma appears to be determined by the interaction between host susceptibility and a variety of environmental exposures. The chemokines are a family of protein mediators with potent chemoattractant activity for granulocytes, monocytes and lymphocytes (Baggiolini et al., 1994). The chemokines are generally subdivided into C-C chemokines having 2 adjacent cysteine residues and into C-X-C chemokines, in which the cysteines are separated by 1 amino acid residue. MCP3 (MIM# 158106) has been reported to interact with several C-C chemokine receptors, which can be simultaneously or selectively expressed on leukocyte subpopulations (Ben-Baruch et al., 1995). The diversity of MCP3 target cell types, as well as the capacity of MCP3 to desensitize leukocyte responses to other C-C chemokines, suggested that MCP3 may interact with multiple C-C chemokine receptors (Xu et al., 1995). MCP3 mRNA was significantly elevated in the bronchial mucosa of atopic and non-atopic asthmatics (Powell et al., 1996) (Humbert et al., 1997), and elevated MCP3 expression was associated with increased numbers of bronchial mucosal eosinophils in the atopic asthmatic patients (Powell et al., 1996). Moreover, MCP3 was significantly increased in bronchoalveolar lavage fluid obtained from asthmatics (Rojas-Ramos et al., 2003), and allergen challenge induced the expression of MCP3 predominantly in the airway

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and related phenotypes. Here, we present four genetic statistical analysis to examine the genetic effects on asthma host genetic study. We performed extensive screening of MCP3 sequencing of the C-C chemokines, little is known about 17q. Despite the characterization of the structure and the sequencing of the C-C chemokines, little is known about genetic polymorphisms and possible involvement in asthma.

In this study, we scrutinized the genetic polymorphisms in MCP3 to evaluate it as a potential candidate gene for asthma host genetic study. We performed extensive screening of MCP3 by direct sequencing to detect polymorphisms and statistical analysis to examine the genetic effects on asthma and related phenotypes. Here, we present four genetic polymorphisms found in MCP3 and the results of an association study in a Korean asthma cohort.

Materials and Methods

Subjects Subjects were recruited from the Asthma Genome Research Center that consists of four tertiary hospitals in Korea (Soonchunhyang University Hospital, Ajou University Hospital, Ulsan University Hospital, and Choong-Ang University Hospital). The DNA was collected from blood samples as described in Kim et al. (2003). Ethical approvals were obtained from the institutional review board of each hospital. All patients had the clinical symptoms and the physical examinations compatible with asthma. Each patient showed airway reversibility as documented by an inhalant bronchodilator-induced improvement of more than 15% of forced expiratory volume in one second (FEV1) and an airway hyperreactivity of less than 10 mg/ml of methacholine. Normal subjects were recruited from spouses of the patients and the general population who answered negatively to a screening questionnaire for respiratory symptoms and had FEV1 greater than 75% of predicted, the provocation concentration causing a fall in the FEV1 for respiratory symptoms and had FEV1 greater than 75% of predicted, the provocation concentration causing a fall in the FEV1 for 20% (PC20) by methacholine greater than 10 mg/ml, and normal finding on a simple chest radiogram. Total IgE was measured using the UniCAP system (Pharmacia Diagnostics, Uppsala, Sweden). Atopy was defined as having wheal reaction by allergen extract equal to or greater than that by histamine (1 mg/ml) or 3 mm in diameter. The clinical parameters are summarized in Table 1.

Sequencing analysis of the human MCP3 gene We have sequenced whole gene (~3 kb), including promoter region, to discover single nucleotide polymorphisms (SNPs) in 24 Korean unregulated individual DNA samples using the ABI PRISM 3700 DNA analyzer (Applied Biosystems, Foster City, USA). Eight primer sets for the amplification and sequencing analysis were designed based on GenBank sequences (Ref. Genome seq.; NT_010799 released on Jan. 2004). Information regarding primers is available on our website (http://www.snp-genetics.com/reference/ 0503_MCP3.doc). Sequence variants were verified by chromatograms. Putative transcription factor binding site was estimated by TFSEARCH (Searching Transcription Factor Binding Sites V1.3, putative score>0.95; http://molsun1.cbrc.aist.go.jp/research/db/ TFSEARCH.html). For comparison of allele frequencies of MCP3 SNPs, which were identified in Korean population, with other major ethnic groups, we also genotyped 50 Caucasian and 50 African-American DNAs obtained from the Human Genetic Cell Repository (http://locus.umdnj.edu/nigms/).

Genotyping with fluorescence polarization detection For genotyping of polymorphic sites with our asthma study, amplifying primers and probes were designed for Taqman (Livak, 1999; Zhang and Li, 2003). Primer Express (Applied Biosystems, Foster City, USA) was used to design both the PCR primers and the MGB TaqMan probes. One allelic probe was labeled with the FAM dye and the other with the fluorescent VIC dye. PCRs were run in TaqMan Universal Master mix without UNG (Applied Biosystems) with PCR primer concentrations of 900 nM and TaqMan MGB-probe concentrations of 200 nM. Reactions were performed in 384-well format in a total reaction volume of 5 ml using 20 ng of genomic DNA. The plates then were placed in a thermal cycler (PE 9700, Applied Biosystems) and heated at 50°C for 2 min and 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min with a final soak at 25°C. The TaqMan assay plates were transferred to the Prism 7900HT instruments (Applied Biosystems) where the fluorescence intensity in each well of the plate was read. Fluorescence data files from each plate were analyzed by automated allele-calling software (SDS 2.1).

Statistics We examined Lewontins D (|D'|) and LD coefficient r² between all pairs of biallelic loci (Hedrick, 1987; Hedrick and Kumar, 2001). Haplotypes of each individual were inferred using the algorithm developed by Stephens et al. (2001), which (PHASE) uses a Bayesian approach incorporating a priori expectations of haplotypic structure from population genetic and coalescent theory. Genetic effects of inferred haplotypes were analyzed in same way as SNPs. Phase probabilities of each site were calculated for each individual by this software. Genotype distribution of MCP3 polymorphisms between the asthmatics and the normal subjects, means and standard deviations (SD) of phenotypes, and P values were calculated by multiple regression analyses controlling age (continuous value), sex (male = 0, female = 1) and smoking status (non-smoker = 0, ex-smoker = 1, smoker = 2) as covariates.
Results and Discussion

Monocye chemotactic protein-3 (MCP3) plays important roles in the pathophysiology in atopic and non-atopic asthma (Ying et al., 1999). Elevated MCP3 mRNA expression was associated with significantly increased numbers of bronchial mucosal eosinophils in the asthmatic patients as compared to the nonasthmatic normal control subjects (Powell et al., 1996).

We identified four sequence variants within the 3 kb full genome including −1,000 bp promoter region of MCP3; one in promoter region (−420T>C), three in intron (+136C>G, +563C>T, +984G>A) respectively. (see Table 2 and Fig. 1). In the estimation of putative transcription factor (TF) binding site, it was shown that four sites for heat-shock transcription factor (HSF) and one for nitrilase-2 (NIT2) (Fig. 1). However, no polymorphism was identified in TF binding site of MCP3.

Those SNPs were genotyped in Korean asthma cohort to...
Byung Lae Park et al. examine the genetic association with asthma and related phenotypes. The frequencies of those four SNPs were 0.020 (-420T>C), 0.038 (+136C>G), 0.080 (+563C>T), 0.035 (+984G>A), respectively, in Korean population (n = 598, Table 2). There were five haplotypes (Table 3) and the significant differences in frequencies of SNPs and haplotypes were observed among three ethnic groups (Table 2 and 3). Two rare SNPs (-420T>C and +136C>G) were not

Linkage disequilibrium coefficients (|D'| and $r^2$) were calculated between all pairs of biallelic loci (Hedrick and Kumar 2001; Hedrick 1987).

### Table 4. Linkage disequilibrium coefficients (|D'|) between SNP loci in MCP3 gene.

| Polymorphisms | -420T>C | +136C>G | +563C>T | +984G>A |
|---------------|---------|---------|---------|---------|
| -420T>C       | -       | 1       | 1       | 1       |
| +136C>G       | $r^2$   | 0.001   | 1       | 1       |
| +563C>T       | 0.002   | 0.003   | -       | 1       |
| +984G>A       | 0.001   | 0.001   | 0.003   | -       |

Linkage disequilibrium coefficients (|D'| and $r^2$) were calculated between all pairs of biallelic loci (Hedrick and Kumar 2001; Hedrick 1987).

### Table 5. Analyses of association of MCP3 polymorphisms with asthma phenotypes (risk of asthma, skin-test reactivity and total serum IgE level) in a Korean asthma cohort.

| Locus | Genotype | Status of Asthma | Skin-test reactivity | Log [Total IgE]** |
|-------|----------|------------------|----------------------|------------------|
|       |          | Asthmatics       | Controls             |                  |
|       |          | (OR (95% CI))    | (OR (95% CI))        |                  |
|       |          | P*               | Negative             | Positive         |
|       |          |                  | OR (95% CI)          |                  |
|       |          |                  | P*                   | N (mean±SD)      |
| -420T>C | TT       | 415 (95.8%)      | 156 (96.9%)          |                  |
|        | CT       | 18(4.2%)         | 4(2.5%)              | 1.16             |
|        | CC       | 1 (0.6%)         | 1 (0.3%)             |                  |
| +136C>G | CC       | 390 (92%)        | 153 (93.3%)          |                  |
|        | CG       | 34 (8%)          | 11 (6.7%)            | 1.45             |
|        | GG       | -                | -                    |                  |
| +563C>T | CC       | 359 (84.9%)      | 136 (84.5%)          |                  |
|        | CT       | 61 (14.4%)       | 24 (14.9%)           | 1.09             |
|        | TT       | 3 (0.7%)         | 1 (0.6%)             |                  |
| +984G>A | GG       | 406 (93.3%)      | 153 (93.3%)          |                  |
|        | AG       | 28 (6.4%)        | 10 (6.1%)            | 0.73             |
|        | AA       | 1 (0.2%)         | 1 (0.6%)             |                  |
| ht1    | -/-      | 15 (3.5%)        | 5 (3.1%)             | 11 (3.4%)        |
|        | ht1/-    | 118 (27.7%)      | 45 (27.4%)           | 0.94             |
|        | ht1/ht1  | 293 (68.8%)      | 114 (69.5%)          |                  |

*P values of co-dominant models
**Analysis among asthmatic patients

Haplotypes were inferred using the algorithm developed by Stephens et al. (2001). Other haplotypes except MCP3-ht1 were not analyzed due to equivalent models with SNPs, e.g., +563C>T=ht2, +136C>G=ht3, +984G>A=ht4 and -420T>C=ht5, respectively. (Table 3).

examine the genetic association with asthma and related phenotypes. The frequencies of those four SNPs were 0.020 (-420T>C), 0.038 (+136C>G), 0.080 (+563C>T), 0.035 (+984G>A), respectively, in Korean population (n = 598,
polymorphic in both Caucasian (n = 50) and African American (n = 50). Linkage disequilibrium coefficients (|D'|) and r² among SNPs were also calculated (table 4). Complete LDs were observed among all SNPs (|D'| = 1) along the gene.

The associations of MCP3 polymorphisms (four SNPs and MCP3-ht1) with risk of asthma, skin-test reactivity, and total serum IgE levels were analyzed. MCP3-ht2, ht3, ht4 and ht5 were not analyzed because they were equivalent with MCP3 +563C>T (MCP3-ht2), MCP3 +136C>G (MCP3-ht3), and MCP3 +984G>A (MCP3-ht4), MCP3 -420T>C (MCP3-ht5) respectively. The statistic analyses are summarized in Table 3. Although 47% of power to detect a disease effect was obtained in this study (calculated by 10% of allele frequency, 450 cases/148 controls, and 2.0 of odds ratio at significance level of 0.01), no significant associations with asthma development or the intermediate phenotypes were detected in analyses (Table 5).

While MCP3 plays critical roles in anaphylactic reactions as interacting with multiple CC chemokine receptors, the genetic polymorphisms of MCP3 might not be associated with asthma development and the intermediate phenotypes (skin test and total serum IgE levels).

In summary, we identified four polymorphisms in the human MCP3 gene by direct sequencing of whole gene and related chemotactic cytokines—CXC and CC chemokines. Adv. Immunol. 55, 97-179.

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