Asthma is a chronic inflammatory disorder of the airways characterized by variable airflow obstruction and bronchial hyperresponsiveness.\(^1,2\) The pathogenesis and etiology of asthma are very complex and not fully understood, although an interaction of multiple genetic loci and a variety of environmental factors have been suggested as important determinants.\(^3-6\) Among them, the promising candidate gene is the cystic fibrosis transmembrane conductance regulator (CFTR) gene that is located on chromosome 7q31.2 (http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene). Mutations in the CFTR gene result in abnormal epithelial ion and water transport and may subsequently incur disturbances in airway mucociliary clearance.

**INTRODUCTION**

Asthma is a chronic inflammatory disorder of the airways characterized by variable airflow obstruction and bronchial hyperresponsiveness.\(^1,5\) The pathogenesis and etiology of asthma are very complex and not fully understood, although an interaction of multiple genetic loci and a variety of environmental factors have been suggested as important determinants.\(^1,6\) Among them, the promising candidate gene is the cystic fibrosis transmembrane conductance regulator (CFTR) gene that is located on chromosome 7q31.2 (http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene).

Mutations in the CFTR gene result in abnormal epithelial ion and water transport and may subsequently incur disturbances in airway mucociliary clearance.
clearance. There are more than 1600 CFTR sequence variations registered in the CF mutation database (http://www.genet.sickkids.on.ca/cftr). However, the majority of mutations have been identified in Caucasians, and furthermore, the spectrum of mutations and genetic polymorphism has not been well described in Asian populations. In Korea, the presentation of classical classic cystic fibrosis (CF) is extremely rare and there are a number of reports regarding this subject.8-10 A few study show that some polymorphisms and mutations of the CFTR gene are associated with respiratory and pancreatic diseases in the Korean population.11,12

The purpose of this study was to evaluate the possible effect of the CFTR gene on susceptibility to asthma in Korean children.

**MATERIALS AND METHODS**

**Subjects**

48 subjects with and without asthma were recruited from Severance Hospital at Yonsei University for this study, comprised of fifty-seven boys and thirty-nine girls.

Asthma diagnosis was made in accordance with the American Thoracic Society (ATS). In short, current asthma was defined as recurrent wheezing or coughing in the absence of a cold in the preceding 12 months with a physician’s diagnosis, and bronchial hyperresponsiveness upon methacholine challenge (PC20 ≤ 16 mg/mL) or at least 12% reversibility of forced expiratory volume in 1 s (FEV1) after inhalation of β2 agonist.13,14 Atopy was defined as a positive skin test to more than one extract of the common local aeroallergens, and non-atopy was defined as a negative skin test and serum IgE concentration less than 100 IU/mL. All subjects were enrolled before the administration of oral or inhaled corticosteroids. Patients treated with systemic corticosteroids due to asthma exacerbation in the preceding 6 months were excluded from this study.

Non-asthma subjects were age-matched to healthy children who visited the hospital for general health workups who had no history of wheezing, recurrent or chronic diseases, infection during the preceding 2 weeks, or hypersensitivity to methacholine. Non-asthma subjects also had negative results on the skin prick test for allergens and did not take any medications.15 All subjects did not have any other disease history including pancreatic diseases. Written consent was obtained from all participants before enrollment in the study, which had been previously approved by the Severance Hospital Institutional Review Board.

**Genotyping**

Whole blood was obtained from each subject and genomic DNA was extracted by using the QIAmp DNA blood Mini kit (QIAGEN, Hilden, Germany) as described.16 The genotyping was analyzed by a single base primer extension assay using a SNaPshot assay kit according to the manufacturer’s protocols (ABI, Foster City, CA, USA), and polymorphisms in the IVS8 TG and T microsatellites were analyzed by bi-directional nucleotide sequencing. Briefly, the genomic DNA region containing both of the single nucleotide polymorphism (SNP) was amplified with PCR reaction. Each PCR reaction contained: 10.0 ng of DNA, 1X PCR Buffer, 0.125 units of AmpliTaq Gold DNA polymerase (ABI), 3.0 mM MgCl2, 0.25 mM of each dNTP, and 0.5 pmole of each primer in 10 µL reaction volume. Reactions were incubated at 95˚C for 10 min, then cycled 30 times at (95˚C for 30 s, 60˚C for 1 min, 72˚C, for 1 min) followed by 72˚C for 5 min.

After amplification, the PCR products were treated with 1 unit each of shrimp alkaline phosphatase (SAP) (Roche) and exonuclease I (USB Corporation) at 37˚C for 60 min and 72˚C for 15 min to purify the amplified products. One microliter of the purified amplification products was added to a SNaPshot Multiplex Ready reaction mixture containing 0.15 pmoles of genotyping primer. The primer extension reaction was carried out for 25 cycles of 96˚C for 10 s, 50˚C for 5 s, and 60˚C for 30 s. The reaction products were treated with 1 unit of SAP at 37˚C for 1 h and 72˚C for 15 min to remove excess fluorescent dye terminators. One microliter of the final reaction samples containing the extension products was added to 9 microliters of Hi-Di formamide (ABI). The mixture was incubated at 95˚C for 5 min, followed by 5 min on ice and then analyzed by electrophoresis in ABI Prism 3730 DNA analyzer. Results were analyzed using Gene Mapper software (ABI).

**Statistical analysis**

Statistical analyses were performed using SPSS 11.5 (SPSS Inc., Chicago, IL, USA). Genotype frequency comparisons between asthma and non-asthma groups were performed by chi-square test. Fisher’s exact test was used if expected cell frequencies were lower than 5. Genotype frequencies at each SNP were tested for Hardy-Weinberg equilibrium. Haplotypes were assembled by using the software based on the Bayesian algorithm (HaploTyper2). All p values were based on two-sided comparisons and p values of less than 0.05 were considered to indicate statistical significance.

**RESULTS**

**Subjects**

The clinical characteristics of the 48 asthma and 48 non-asthma subjects are presented in Table 1. There were no
statistical differences in demographic data such as age and sex between the two groups. However, subjects with asthma were significantly associated with lower lung function ($p < 0.05$). In addition, there were significant differences in total eosinophil counts, total IgE, and serum eosinophil cationic protein (ECP) with atopy-related parameters between the asthma and non-asthma group ($p < 0.01$).

**Genotype frequencies in asthma and non-asthma groups**

To investigate the association between CFTR genetic variations and asthma, a case-control study was performed using samples from 98 subjects as detailed in Materials and Methods. We genotyped the 14 mutations identified in Korea as summarized in Table 2.*11 Diallelic loci were analyzed by automated DNA screening (SNPshot; Applied Biosystems Inc.), and the TGn, Tn numbers were identified by bi-directional nucleotide sequencing. Among the 14 mutations, there are no mutant variants in Q98R, I125T, A309, Q220X, and Q1291X loci in our sample and the genotype frequencies of the remaining variants are listed in Table 3. There were no significant differences in genotype frequencies in asthma and non-asthma groups.

### Table 1. Clinical Characteristics of the Study Subjects

| Characteristics | Asthma (n = 48) | Non-asthma (n = 48) | $p$ value* |
|-----------------|-----------------|---------------------|------------|
| Age (yrs; mean ± SD) | 9.48 ± 2.04 | 9.63 ± 2.44 | 0.753 |
| Sex [Male; n (%)] | 33 (71.7) | 24 (50.0) | 0.037 |
| **Lung Function** | | | |
| FVC (% predicted; mean ± SD) | 83.50 ± 10.11 | 89.18 ± 9.88 | 0.016 |
| FEV1 (% predicted; mean ± SD) | 77.47 ± 18.98 | 86.54 ± 10.54 | 0.010 |
| FEV1/FVC (% predicted; mean ± SD) | 97.91 ± 8.26 | 105.74 ± 5.87 | < 0.001 |
| FEF25-75 (% predicted; mean ± SD) | 70.34 ± 20.89 | 89.45 ± 24.76 | 0.001 |
| PEF (% predicted; mean ± SD) | 78.92 ± 21.49 | 91.56 ± 27.93 | 0.028 |
| Methacholine PC20 [mg/mL; n (%)] | < 16 | 48 (100) | 0 (0) | < 0.001 |
| ≥ 16 | 0 (0) | 48 (100) | |
| Total serum IgE levels (ln IU/mL; mean ± SD) | 5.63 ± 1.84 | 3.26 ± 1.23 | < 0.001 |
| Total Eosinophil count (ln µL-1; mean ± SD) | 6.27 ± 0.67 | 4.92 ± 0.91 | < 0.001 |
| Eosinophil cation protein (ln µg/L; mean ± SD) | 3.73 ± 1.75 | 2.44 ± 1.22 | < 0.001 |

*FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; FEF, forced expiratory flow; FEF25-75, forced expiratory flow between 25% and 75% of the FVC; PEF, peak expiratory flow. *χ2 test or t-test were used where appropriate.

### Table 2. CFTR Genetic Variations Analyzed in This Study

| Name | Nucleotide change | Exon | Consequence | Reference |
|------|------------------|------|-------------|-----------|
| - 8G / C | G to C at 125 | 5' UTR | sequence variation | 9 |
| Q98R | A to G at 425 | Exon 4 | Glu to Arg at 98 | 8 |
| I125T | T to C at 506 | Exon 4 | Ile to Thr at 125 | 9 |
| E217G | A to G at 782 | Exon 6a | Glu to Gly at 217 | 9 |
| Q220X | C to T at 790 | Exon 6a | Glu to Stop at 220 | 7, 8 |
| A309A | C or G at 1059 | Exon 7 | Sequence variation | 9 |
| TG repeat | TGn10-13 | IVS 8 | Splicing | 9 |
| T repeat | Tn | IVS 8 | Splicing | 9 |
| M470V | A or G at 1540 | Exon 10 | Met to Val at 470 | 9 |
| I556V | A to G at 1798 | Exon 11 | Ile to Val at 556 | 9 |
| T854T | C to G at 2694 | Exon 14a | Sequence variation | 9 |
| Q1291X | C to T at 4003 | Exon 20 | Glu to Stop at 1291 | 9 |
| Q1352H | G to C at 4188 | Exon 22 | Glu to His at 1352 | 9 |
| R1453W | C to T at 4489 | Exon 24 | Arg to Trp at 1453 | 9 |

CFTR, cystic fibrosis transmembrane conductance regulator.

Mutation names and nucleotide numbers are presented according to the Cystic Fibrosis Genetic Analysis Consortium (CFGAC) (http://www.genet.sickkids.on.ca/).
and allele frequencies of the 9 polymorphisms observed between the non-asthma and asthma groups.

**Haplotype patterns and their disease associations**
Since multiple alleles were analyzed in our study, a haplotype-based approach was applied to find the disease-associated CFTR variations. The Haplotype program based on the Bayesian algorithm was used and haplotypes were assembled using the genotype data obtained from the 98 tested samples. Nineteen loci consisting of 7 diallelic variants and two microsatellites of IVS8 TG, and T were analyzed. Since the program accepts only diallelic data, IVS8 TG, TG10, and TG11 were considered as wild-type (WT), and TG12 or TG13 were regarded as mutant. For IVS8 T, T was considered mutant and other alleles were applied as WT.

After 100 rounds of interactions, 8 haplotypes were assembled and their identification (ID) numbers were assigned according to the total sample frequencies (Table 4). Major haplotypes showing over 1% frequency in both groups are presented in this table. Differences between non-asthma and asthma groups were analyzed by the chi-square analysis. However, no significant differences were found in haplotype frequencies between the two groups.

### DISCUSSION
This is the first study to investigate the association between CFTR mutations and asthma in Korean children, and no significant association was found in our pilot study. However, the association between CFTR mutations and asthma is controversial. Mennie, et al. did not find any association between the CFTR gene mutations and asthma in a British population. The lack of significant association between CF heterozygosity and asthma found in the present study is also supported by studies from the French, Italian, Singaporean Chinese, and Norwegian populations. Furthermore, Hakonarson, et al. demonstrated that

| Variants      | Non-asthma (n) | Asthma (n) | p value* |
|---------------|----------------|------------|----------|
| - 8G / C      | 39             | 37         | 0.466    |
| G / C         | 8              | 11         |          |
| C / C         | 1              | 0          |          |
| E217G         | 48             | 46         | 0.247    |
| A / G         | 0              | 2          |          |
| M470V         | 8              | 10         | 0.858    |
| A / G         | 25             | 23         |          |
| G / G         | 15             | 15         |          |
| I556V         | 42             | 45         | 0.276    |
| A / G         | 4              | 3          |          |
| T854T         | 15             | 16         | 0.639    |
| T / G         | 26             | 22         |          |
| G / G         | 7              | 10         |          |
| Q1352H        | 46             | 46         | 0.383    |
| G / C         | 2              | 2          |          |
| R1453W        | 47             | 46         | 0.500    |
| C / T         | 0              | 1          |          |
| Microsatellite|                |            |          |
| TG repeat (IVS 8) | 10 | 12 | 0.119 |
| W / W         | 27             | 18         |          |
| M / M         | 10             | 18         |          |
| T repeat (IVS 8) | 6 | 7 | 1 0.141 |
| 5 / 7         | 2              | 1          |          |
| 6 / 7         | 0              | 1          |          |
| 7 / 7         | 44             | 42         |          |
| 7 / 9         | 1              | 4          |          |

*Variant frequencies were obtained by using the chi-square test and Fisher’s exact test (expected cell value < 5) and the Q98R, I125T, A309, Q220X, and Q1291X variants were excluded from the table because of no frequency. TG10 and TG11 were regarded as wild-type (W) and TG12 and TG13 were regarded as mutant-type (M).
a study from Iceland failed to show evidence of a linkage between asthma and chromosome 7q31.2.

In contrast, Dahl, et al.\textsuperscript{22} found that ∆F508 heterozygosity was associated with an increased susceptibility to asthma in a Danish population. Additionally, studies from Greek\textsuperscript{23,24} and Spanish\textsuperscript{25} populations reported a positive association between asthma and CF heterozygosity.\textsuperscript{24} Schreuder, et al.\textsuperscript{26} suggested that obligate ∆F508 carriers are protected from asthma. However the background haplotype for ∆F508,\textsuperscript{27} which accounts for 66% of worldwide cystic fibrosis, is very rare in the Korean population.\textsuperscript{11} Besides, genetic variants at Q1352H or E217G were found to be associated with bronchiectasis and/or chronic pancreatitis in the Korean population.\textsuperscript{11} In particular, non-synonymous Q1352H and E217G mutations in the M470 background caused a 60-80% reduction in CFTR-dependent Cl⁻ currents and HCO₃⁻ transport activities. However, we could not find any significant association at those sites in this study. In addition, Q220X and Q1291X mutations that give rise to premature stop codon can lead to aberrant function. However, there are no mutant variants in those loci in our study sample.

Several reports suggested that ∆F508 carriers have lower values of pulmonary function such as FEV₁ or FVC compared to non-carriers, although no difference in the annual decline in lung function was observed between the two groups.\textsuperscript{24,28} However, Byard and Davis\textsuperscript{29} showed that there are no significant differences in spirometric values between CFTR gene mutation carriers and non-carriers. In this study, we did not have any significant correlation between spirometric values and CFTR gene mutations in the 14 mutations (Table 2, data not shown).

It is worth considering some limitations of our study. The sample size was too small and we did not investigate the full sequence of the CFTR gene. Further study is recommended to verify the results based on our pilot study.

We conclude that this study has failed to produce evidence in support of the notion that CFTR genetic variations identified in the Korean population significantly influences the expression of the asthmatic phenotype.

### ACKNOWLEDGEMENTS

This study was supported by a faculty research grant of Yonsei University College of Medicine for 2007 (6-2007-0112), the Korea Health 21 R&D Project, Ministry for Health, Welfare and Family Affairs, R.O.K. (A030001), and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0001664).

### REFERENCES

1. Maselli R, Paciocco G. Asthma: pathophysiology of the bronchial obstruction. Allergy 2000;55 Suppl 61:49-51.
2. Shin JW, Sue JH, Song TW, Kim KW, Kim ES, Sohn MH, et al. Atopy and house dust mite sensitization as risk factors for asthma in children. Yonsei Med J 2005;46:629-34.
3. Maddox L, Schwartz DA. The pathophysiology of asthma. Annu Rev Med 2002;53:477-98.
4. Sengler C, Lau S, Wahn U, Nickel R. Interactions between genes and environmental factors in asthma and atopy: new developments. Respir Res 2002;3:7.
5. Nam HS, Lee SY, Kim SJ, Kim JS, Kwon SS, Kim YK, et al. The soluble tumor necrosis factor-alpha receptor suppresses airway inflammation in a murine model of acute asthma. Yonsei Med J 2009;50:569-75.
6. Kim SH, Ye YM, Hur GY, Lee HY, Lee YK, Lee SH, et al. Effect of beta2-adrenergic receptor polymorphism in asthma control of patients receiving combination treatment. Yonsei Med J 2009;50:182-8.
7. Munthe-Kaas MC, Lødrup Carlsen KC, Carlsen KH, Skinningsrud B, Håland G, Devulapalli CS, et al. CFTR gene mutations and asthma in the Norwegian Environment and Childhood Asthma study. Respir Med 2006;100:2121-8.
8. Moon HR, Ko TS, Ko YY, Choi JH, Kim YC. Cystic fibrosis--a case presented with recurrent bronchiolitis in infancy in a Korean male infant. J Korean Med Sci 1988;3:157-62.
9. Ahn KM, Park HY, Lee JH, Lee MG, Kim JH, Kang JI, et al. Cystic fibrosis in Korean children: a case report identified by a quantitative pilocarpine iontophoresis sweat test and genetic analysis. J Korean Med Sci 2005;20:153-7.
10. Koh WJ, Ki CS, Kim JW, Kim JH, Lim SY. Report of a Korean patient with cystic fibrosis, carrying Q98R and Q220X mutations in the CFTR gene. J Korean Med Sci 2006;21:563-6.
11. Lee JH, Choi JH, Namkung W, Hanrahan JW, Chang J, Song SY, et al. A haplotype-based molecular analysis of CFTR mutations associated with respiratory and pancreatic diseases. Hum Mol Genet 2003;12:2321-32.
12. Lee KH, Ryu JK, Yoon WJ, Lee JK, Kim YT, Yoon YB. Mutation analysis of SPINK1 and CFTR gene in Korean patients with alcoholic chronic pancreatitis. Dig Dis Sci 2005;50:1852-6.
13. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, et al. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. Am J Respir Crit Care Med 2000;161:309-29.
14. Sedgwick JB, Vrtil RF, Jansen KJ, Kita H, Bartemes K, Busse WW. Peripheral blood eosinophils from patients with allergic asthma contain increased intracellular eosinophil-derived neurotoxin. J Allergy Clin Immunol 2004;114:568-74.
15. Tang K, Ngoi SM, Gwee PC, Chua JM, Lee EJ, Chong SS, et al. Distinct haplotype profiles and strong linkage disequilibrium at the MDRI multidrug transporter gene locus in three ethnic Asian populations. Pharmacogenetics 2002;12:437-50.
16. Niu T, Qin ZS, Xu X, Liu JS. Bayesian haplotype inference for multiple linked single-nucleotide polymorphisms. Am J Hum Genet 2002;70:157-69.
17. Mennie M, Gilfillan A, Brock DJ, Liston WA. Heterozygotes for the delta F508 cystic fibrosis allele are not protected against bronchial asthma. Nat Med 1995;1:978-9.
18. de Cid R, Chornel JC, Lazaro C, Sunyer J, Baudis M, Casals T, et al. CFTR and asthma in the French EGEA study. Eur J Hum Genet 2001;9:67-9.
19. Castellani C, Quinzii C, Altieri S, Mastella G, Assael BM. A pilot survey of cystic fibrosis clinical manifestations in CFTR mutation heterozygotes. Genet Test 2001;5:249-54.
20. Ngiam NS, Chong SS, Shek LP, Goh DL, Ong KC, Chng SY, et al. Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations in Asians with chronic pulmonary disease: a pilot study. J Cyst Fibros 2006;5:159-64.
21. Hakonarson H, Bjornsdottir US, Ostermann E, Amason T, Adalsteinsdottir AE, Halapi E, et al. Allelic frequencies and patterns of single-nucleotide polymorphisms in candidate genes for asthma and atopy in Iceland. Am J Respir Crit Care Med 2001;164:2036-44.
22. Dahl M, Tybjaerg-Hansen A, Lange P, Nordestgaard BG. DeltaF508 heterozygosity in cystic fibrosis and susceptibility to asthma. Lancet 1998;351:1911-3.
23. Tzetis M, Efthymiadou A, Strofalis S, Psychou P, Dimakou A, Pouliou E, et al. CFTR gene mutations--including three novel nucleotide substitutions--and haplotype background in patients with asthma, disseminated bronchiectasis and chronic obstructive pulmonary disease. Hum Genet 2001;108:216-21.
24. Douros K, Loukou I, Doudounakis S, Tzetis M, Priftis KN, Kanavakis E. Asthma and pulmonary function abnormalities in heterozygotes for cystic fibrosis transmembrane regulator gene mutations. Int J Clin Exp Med 2008;1:345-9.
25. Lázaro C, de Cid R, Sunyer J, Soriano J, Giménez J, Alvarez M, et al. Missense mutations in the cystic fibrosis gene in adult patients with asthma. Hum Mutat 1999;14:510-9.
26. Schroeder SA, Gaughan DM, Swift M. Protection against bronchial asthma by CFTR delta F508 mutation: a heterozygote advantage in cystic fibrosis. Nat Med 1995;1:703-5.
27. Cuppens H, Teng H, Raeymaekers P, De Boeck C, Cassiman JJ. CFTR haplotype backgrounds on normal and mutant CFTR genes. Hum Mol Genet 1994;3:607-14.
28. Dahl M, Nordestgaard BG, Lange P, Tybjaerg-Hansen A. Fifteen-year follow-up of pulmonary function in individuals heterozygous for the cystic fibrosis phenylalanine-508 deletion. J Allergy Clin Immunol 2001;107:818-23.
29. Byard PJ, Davis PB. Pulmonary function in obligate heterozygotes for cystic fibrosis. Am Rev Respir Dis 1988;138:312-6.