

Variant in Promoter Region of Platelet-Derived Growth Factor Receptor- α (PDGFR α) Gene Is Associated with the Severity and Allergic Status of Childhood Asthma

Lawrence Shih-Hsin Wu^a Choon-Yee Tan^b Ling-Mei Wang^d
Cherry Guan-Ju Lin^a Jiu-Yao Wang^{b, c}

^aResearch Development Division, Vita Genomics Inc., Wugu, Taipei County, ^bInstitute of Molecular Medicine, and

^cDepartment of Pediatrics, College of Medicine, National Cheng Kung University, Tainan, and

^dDivision of Molecular and Genomic Medicine, National Health Research Institute, Zhunan, Miaoli County, Taiwan

Key Words

Platelet-derived growth factor receptor- α • Genetic polymorphisms • Asthma, childhood • Airway remodeling

Abstract

Background: Upregulation of the platelet-derived growth factor receptor- α (PDGFR α) in airway myofibroblast cells is one of the mechanisms of airway remodeling. The genetic association between PDGFR α promoter polymorphism and severity of childhood asthma was examined. **Methods:** Five single nucleotide polymorphisms (SNPs) at the promoter regions of the PDGFR α gene were genotyped in 277 unrelated allergic and nonallergic asthmatic children and 93 age-matched controls. Promoter haplotypes were constructed using SNP genotyping data. The serum level of PDGF-AA, the ligand for PDGFR α , was assayed by ELISA kits. **Results:** The genotype distribution of SNP rs1800810 (–1171G/C) in nonallergic asthma was significantly different from controls ($p = 0.038$), as well as its allele distribution ($p = 0.028$). Using haplotype analysis, the combination frequency of the low expression of H1 homozygous and heterozygous genotype (H1/H1 + H1/H2) was significantly higher in nonallergic asthma as compared to controls (OR = 1.94, CI = 1.11–3.39, $p < 0.02$). The frequency of H2/H2 homozygous was higher in

persistent asthma than in intermittent asthma ($p = 0.008$, OR = 2.625). In addition, the PDGF-AA serum level in H2/H2 homozygous haplotype was significantly lower as compared to non-H2/H2 homozygous haplotype both in asthmatic (138.1 ± 62.9 vs. 249.7 ± 97.1 ng/ml, $p < 0.05$) and nonallergic asthmatic children (113.8 ± 38.0 vs. 256.6 ± 58.3 ng/ml, $p < 0.05$). **Conclusions:** The developmental deficiency due to the low expression of PDGFR α may be one of the susceptible factors for nonallergic asthmatic children. There was also an autocrine effect of lower PDGF-AA and higher PDGFR α expression that might lead to airway remodeling causing the severity of asthma. Copyright © 2006 S. Karger AG, Basel

Introduction

Asthma is a chronic inflammatory disease of the bronchial airways orchestrated by Th2 cells and their secreted cytokines [1]. The long-term effect of this chronic airway inflammation leads to excessive airway mucus production, pulmonary eosinophilia, bronchial epithelia damage, airway hyperresponsiveness, and airway remodeling marked by increases in airway smooth muscle mass and peribronchiolar collagen deposition [2]. The precise

mechanism underlying the initiation and development of airway remodeling, however, is not fully understood. Increasing evidence suggests that the growth factors, which contributed to mediating lung fibroblast proliferation, such as transforming growth factor- β (TGF- β), insulin-like growth factor-1, platelet-derived growth factor (PDGF) isoforms, fibroblast growth factors (FGFs), and members of the epidermal growth factor family, are found responsible in airway remodeling of asthma [3].

PDGF is an important cytokine in fibrogenic processes. It is a 30-kDa homodimer or heterodimer of two disulfide-linked A or B peptides (PDGF-AA, PDGF-AB, and PDGF-BB), and is expressed by a variety of cell types, particularly activated monocytes and alveolar macrophages in the lung [4]. PDGFs activate their receptors by promoting formation of receptor dimers. PDGF-A chain binds selectively to the platelet-derived growth factor receptor- α (PDGFR α), while PDGF-B chain isoforms bind and dimerize both PDGFR α and PDGFR β [5]. PDGF receptors contain an extracellular loop for association of the ligand, a transmembrane domain and an intracellular tail with a tyrosine kinase domain [6]. From several studies in knockout mice, it is clear that signaling through PDGFR α is important in the normal development of several tissues including pulmonary smooth muscle cells [7].

The genetic polymorphisms of platelet-derived growth factor α -receptor gene (PDGFRA) play important roles in the susceptibility for neural tube defects [8]. It was suggested that the upregulation of PDGFR α by FGF- β , IL-1 β and TGF- β contributes to cellular hyperplasia of bronchial smooth muscles during chronic airway inflammation in asthma [9]. Using short tandem repeats (STR) markers in whole genome linkage disequilibrium mapping, we found that allele frequencies of several STR markers were significantly associated with childhood asthma [10], in which the PDGFRA gene is located at chromosome 4q12 between two statistically significant STR markers, D4S2971 and D4S1592, found in earlier reports by us [10, 11].

In this study, we identified the polymorphisms at the PDGFRA promoter region in primary school children in Taiwan. When comparing the phenotypes, allergic and nonallergic, of childhood asthma and their asthma severity, we found that different haplotypes of genetic polymorphisms at the promoter region of the PDGFRA gene were associated with the nonallergic type of asthma and the severity of childhood asthma, respectively. These results further imply that childhood asthma is a heterogeneous group composed of different susceptibility genes in allergic and nonallergic asthma.

Material and Methods

Subjects

Our study population consisted of 277 allergic and nonallergic asthmatic children, aged between 6 and 13 years and 93 age-matched controls, who were randomly selected on the basis of a childhood allergy and asthma questionnaire survey conducted in 5,000 schoolchildren from 5 primary schools in Tainan City, Taiwan. All participants or their guardians, being well-informed about the study protocol, signed the consent forms, and filled in a modified Chinese version of the International Study of Asthma and Allergies in Childhood (ISAAC-C) questionnaire, as well as answered additional questions pertinent to the diagnosis and assessment of asthma [12]. The Ethical and Clinical Trial Committee of the National Cheng-Kung University Hospital approved this study protocol. The diagnosis and classification of the clinical severity of asthma was made according to the GINA guidelines [13]. The definition of asthma included (1) a history of two or more episodes of wheezing in the preceding 6 months, during or without concurrent respiratory infections, (2) a chronic cough for more than 1 month and a diagnosis by a physician of the presence of wheezing episode(s), and (3) a bronchodilator test confirming the positive response of increased 15% of FEV₁. Age-matched controls did not have any of the above conditions at their previous physical checkup and past history. Other evaluations included skin prick tests for responsiveness to 6 common aeroallergens, a differential blood count (including total eosinophil count), and measures of total serum IgE, as well as specific IgE to house dust and mixed pollens using the UniCAP system (Pharmacia Diagnostic, Sweden). A positive skin test was defined as the presence of $\geq 1+$ reaction or with a wheal diameter ≥ 5 mm. The confirmed asthmatic children were further divided by their serum IgE levels into an allergic group (140 subjects) that had high IgE serum concentrations ($\geq 1,000$ IU/ml) and were sensitized to at least one aeroallergen, and a nonallergic group (137 subjects) with IgE serum levels within the normal range (≤ 200 IU/ml) and no detectable aeroallergen sensitization.

DNA Preparation

Genomic DNAs were extracted from 370 unrelated Taiwanese children (93 healthy controls, 140 with allergic asthma, and 137 with nonallergic asthma). DNA was isolated from blood samples using QIAamp DNA Blood kit according to the manufacturer's instructions. The isolated genomic DNA was quality checked by agarose gel electrophoresis analysis, quantity determined spectrophotometrically, and stored at -80°C .

Single Nucleotide Polymorphism Genotyping

The DNA fragment flanking the promoter region of the PDGFRA gene was amplified by the ABI 2700 PCR machine using two pairs of forward and reverse primers. The sequence and related information of primers are listed in table 1. The fragments of the PCR product were sequenced by the ABI 3700 automatic sequencer according to the manufacturer's protocol. The sequence data were analyzed by PolyPhred software to identify the potential candidate single nucleotide polymorphisms (SNPs). The potential SNPs were manually checked to ensure the presence of true SNPs and the allele of each individual. There was independent manual confirmation for all the sequence data and only the confirmed data were subjected to the subsequent statistical analysis.

Table 1. PCR primers used to detect polymorphisms of the promoter region for PDGFRA

Primer sequence (5' to 3')	Region of interest	Polymorphism site	SNP ID	Annealing temperature, °C	Product size, bp
Forward: TTTTGCCTTTTATTCCGCAC Reverse: AGTGTCGGGAGGAAACGAT	promoter	-1288	rs1800809	60	626
		-1247	rs6554162		
		-1171	rs1800810		
Forward: ACTCTGCCCAGGTCTGGTTC Reverse: GTCCCTTCTACAAACTCGGC	promoter	-735	rs1800813	60	601
		-573	rs1800812		

Table 2. Baseline characteristics of the study population

Characteristics	Allergic asthma (n = 140)	Nonallergic asthma (n = 137)	Controls (n = 93)
Age ^a , years	8.2 ± 3.3	6.8 ± 3.7	7.9 ± 1.6
Males	97 (69.3)	87 (63.5)	51 (54.8)
Asthma (doctor's diagnosis), %	100	100	0
Predicted FEV ₁ ^a , %	88.2 ± 12.2	91.3 ± 11.7	114.3 ± 13.2
FEV ₁ /FVC ^a , %*	85.3 ± 9.8	92.7 ± 7.6	112.8 ± 9.8
Severity of asthma			ND
I (mild, intermittent)	5 (4.76)	26 (30.59)	
II (mild, persistent)	11 (10.48)	22 (25.88)	
III (moderate, persistent)	28 (26.67)	19 (22.35)	
IV (severe, persistent)	30 (28.57)	7 (8.24)	
Unknown	31 (29.52)	11 (12.94)	
Skin test positive for Der p mite extracts	68 (94.4)	0	7 (7.3)
Serum total IgE ^a , IU	2,756.5 ± 1,901.5	90.3 ± 56.9	43.7 ± 24.3

Values in parentheses represent percentage. * p < 0.05.
^a Values represent mean ± SD.

PDGF-AA Enzyme Immunoassay

PDGF-AA concentrations in the sera of different haplotypes of PDGFRA gene polymorphisms were measured by the quantitative sandwich enzyme immunoassay technique (Human PDGF-AA Quantikine ELISA Kit, R&D Systems, Minneapolis, Minn., USA), according to the manufacturer's manual. The minimum detectable dose of PDGF-AA ranged from 0.74 to 10.1 pg/ml.

Statistical Analysis

The primary dichotomous outcome variable of association analyses was the control or case status for asthma (i.e. allergic asthma with high IgE, nonallergic asthma with normal IgE, or both). Four grades of asthma severity were grouped and combined into two categories, that is intermittent (grade 1) and persistent (grades 2, 3 and 4), and analyzed in the second part. The principal explanatory variables were the genotyped polymorphism. All statistical analyses, including χ^2 tests, odds ratios (OR), and Fisher's exact test, were performed with the SAS soft-

ware (Cary, N.C., USA). Promoter haplotypes were constructed using SNP genotyping data of the Hap web site (<http://www1.cs.columbia.edu/compbio/hap/>).

Results

Characteristics of Patients

Three hundred and seventy DNA samples extracted from asthmatic children and age-matched controls were collected for genotyping. In table 2, the average age for allergic asthma and nonallergic asthma was 8.2 ± 3.3 and 6.8 ± 3.7 years, respectively. The mean ages and the ratio of gender in each group did not show a significant difference. In our study population, the severity of asth-

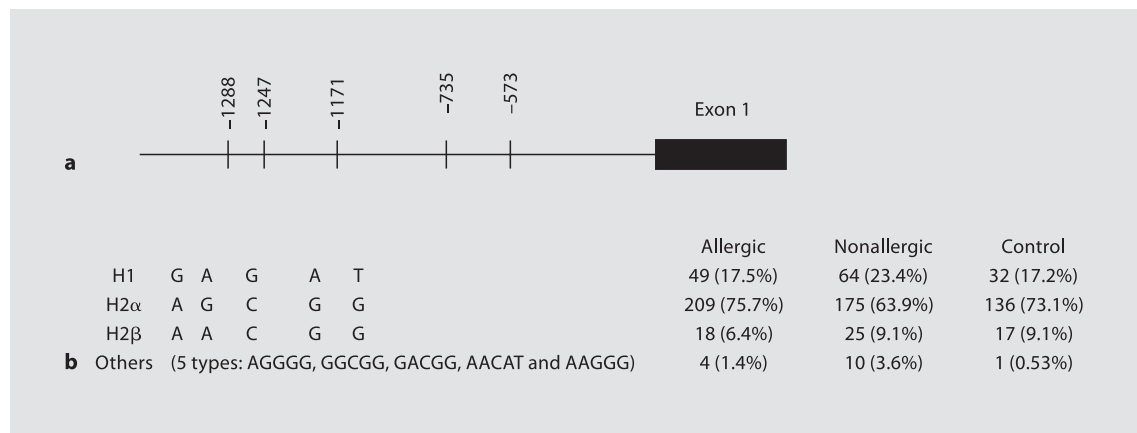


Fig. 1. Promoter haplotypes resulting from polymorphisms in the promoter region of PDGFRA. **a** The identified five polymorphic sites are indicated together with their corresponding nucleotide position (RefSeq nucleotide NM_006206). **b** The three major haplotypes constructed by the Hap web site are shown on the left together with their nucleotide sequence at polymorphic sites. Haplotype frequencies in controls and groups of allergic and nonallergic asthmatics are shown on the right.

ma (four grades according to the GINA guidelines) was significantly associated with the IgE level ($p < 0.001$, $2 \times 4 \chi^2$ test, 74 missing/unknown data).

Allele and Genotype Frequencies of the SNPs in PDGFRA Promoter Region Were Different between Control and Nonallergic Asthmatics

The SNP genotyping revealed five polymorphic sites and three major haplotypes, H1, H2α, and H2β, in the 2-kb region upstream of the transcription initiation site of PDGFRA in our study subjects (fig. 1). Four of these five SNP sites, except G-1274A, were nearly in complete association, which involved in the common consensus-binding sites for GCF (AGCGGCGGGA, -899 to -908) and SP1 transcription factors (AAGCGGCGGG, -900 to -909), as reported by others [14]. Table 3 lists the frequencies of alleles (table 3a) and genotypes (table 3b) of these 5 SNP polymorphism sites in asthmatic and control children. The genotype distribution of SNP rs1800810 (-1171G/C) in nonallergic asthma was significantly different from controls ($p = 0.038$) as well as its allele distribution ($p = 0.028$). The genotype distribution of SNP rs1800809 (-1288 A/G) in nonasthma was also significantly different from controls ($p = 0.039$), but not to a significant difference of allele frequency as compared to controls ($p = 0.053$). There were no significant differences of genotype and allele frequencies at these two SNP sites between allergic asthma and controls, or in allergic

and nonallergic asthma children. The other three SNPs, rs6554162 (-1247G/C), rs1800813 (-735G/A), and rs1800812 (-573G/T), did not reveal any difference in genotype and allele frequencies among these three groups.

In the combination of these 5 SNP sites, we also found three major haplotypes, H1 and two H2 variants (α and β), which were designated by Joosten et al. [8], in our study population (fig. 1). The overall haplotype distribution in the allergic asthmatics was similar to that of the control group (fig. 1), while the nonallergic asthmatics showed an increase in H1 haplotype frequency combined with a reduction of H2α haplotype, which did, however, not reach statistical difference ($p > 0.05$).

H1 Haplotype Was Associated with Nonallergic Asthma

The various promoter haplotypes of PDGFRA may contribute different transcriptional activities. It has been reported that H2α and H2β haplotypes have up to a 6-fold higher activity than haplotype H1 [8]. In order to conveniently compare the expression level of PDGFRA gene between groups, we combined the H2α and H2β as a high expression H2 haplotype. The allele frequencies of high (H2) and low (H1) expression haplotype variants were different between control and nonallergic asthmatics, but did not reach a significant level

Table 3. Allele frequencies and genotype frequencies of the SNPs in promoter region of the PDGFRA gene in studied subjects**a** Allele frequencies

SNP ID	Allele	Allele frequency			p value		
		controls	allergic asthma	nonallergic asthma	C vs. AA + NA	C. vs. AA	C vs. NA
rs1800809	A	154 (82.8%)	227 (82.2%)	203 (75.2%)	0.236	0.879	0.053
	G	32 (17.2%)	49 (17.8%)	67 (24.8%)			
rs6554162	G	136 (73.1%)	209 (75.7%)	182 (66.4%)	0.144	0.258	0.127
	A	50 (26.9%)	97 (24.3%)	92 (33.6%)			
rs1800810	G	154 (82.8%)	223 (80.8%)	203 (74.1%)	0.123	0.587	0.028*
	C	32 (17.2%)	53 (19.2%)	71 (25.9%)			
rs1800813	G	149 (81.9%)	229 (82.4%)	208 (76.5%)	0.48	0.889	0.169
	A	33 (18.1%)	49 (17.6%)	64 (23.5%)			
rs1800812	G	151 (82.1%)	231 (82.5%)	211 (76.4%)	0.449	0.904	0.149
	T	33 (17.9%)	49 (17.5%)	65 (23.6%)			

b Genotype frequencies

SNP ID	Genotype	Genotype frequency			p value		
		controls	allergic asthma	nonallergic asthma	C vs. AA + NA	C vs. AA	C vs. NA
rs1800809	AA	65 (69.9%)	90 (65.2%)	73 (54.1%)	0.072	0.094	0.039*
	AG	24 (25.8%)	47 (34.1%)	57 (42.2%)			
	GG	4 (4.3%)	1 (0.7%)	5 (3.7%)			
rs6554162	GG	51 (54.8%)	77 (55.8%)	55 (40.2%)	0.229	0.402	0.055
	AG	34 (36.6%)	55 (39.9%)	72 (52.6%)			
	AA	8 (8.6%)	6 (4.4%)	10 (7.3%)			
rs1800810	GG	65 (69.9%)	88 (63.8%)	73 (53.3%)	0.111	0.308	0.038*
	CG	24 (25.8%)	47 (34.1%)	57 (41.6%)			
	CC	4 (4.3%)	3 (2.2%)	7 (5.1%)			
rs1800813	GG	62 (68.1%)	91 (65.5%)	76 (55.9%)	0.111	0.114	0.093
	AG	25 (27.5%)	47 (33.8%)	56 (47.2%)			
	AA	4 (4.4%)	1 (0.7%)	4 (2.9%)			
rs1800812	GG	63 (68.5%)	92 (65.7%)	77 (55.8%)	0.102	0.121	0.076
	GT	25 (27.2%)	47 (33.6%)	57 (41.3%)			
	TT	4 (4.4%)	1 (0.7%)	4 (2.9%)			

* $p < 0.05$. C = Control; AA = allergic asthma subjects with high IgE; NA = nonallergic asthma subjects with normal IgE.

(table 4). The H2/H2 genotype frequency of nonallergic asthmatics showed 25% reduction as compared with control subjects (table 4a). The combination frequency of H1 homozygous and heterozygous genotype (H1/H1 + H1/H2) in nonallergic asthmatics was significantly higher in nonallergic asthma as compared to controls (OR = 1.94, CI = 1.11–3.39, $p < 0.02$) (table 4b), while there was no significant difference in allele or genotype frequencies of *PDGFRA* promoter haplotypes between control and allergic asthmatics.

Genotype and Haplotype Frequencies of SNPs in PDGFRA Promoter Region Were Associated with the Severity of Asthma

When the severity of asthma was considered, the genotype frequencies of these 5 SNPs in the *PDGFRA* promoter region all showed significant differences between intermittent and persistent asthma (data not shown). The frequencies of major homozygous genotypes in each SNP site tested, such as –1288A/A, –1247G/G, –1171G/G, –735G/G and –573G/G, were all higher in persistent asthma than in intermittent asthma, respectively. When the

Table 4. Genotype frequencies of haplotypes in the promoter region of the PDGFRA gene in studied subjects and comparisons of allele and genotype frequencies for PDGFRA promoter haplotypes

a Genotype frequencies of haplotypes in the promoter region of the PDGFRA gene

Genotype	Frequency		
	controls	allergic asthma	nonallergic asthma
H1	32 (17.3%)	49 (17.8%)	64 (24.2%)
H2	153 (82.7%)	227 (82.2%)	200 (75.8%)
H1H1	4 (4.3%)	1 (0.7%)	4 (2.9%)
H1H2	24 (25.8%)	47 (33.6%)	56 (41.3%)
H2H2	64 (68.8%)	90 (64.3%)	72 (52.2%)
Others	1 (1.1%)	2 (1.4%)	5 (3.6%)

b Comparisons of allele and genotype frequencies for PDGFRA promoter haplotypes

	p value		OR (95% CI)	
	C vs. AA	C vs. NA	C vs. AA	C vs. NA
H1/H2	0.9	0.08	1.03 (0.63–1.69)	1.53 (0.95–2.46)
H1H1 + H1H2/H2H2	0.49	0.02*	0.82 (0.47–1.44)	1.94 (1.11–3.39)

* $p < 0.05$. C = Control; AA = allergic asthma subjects with high IgE; NA = nonallergic asthma subjects with normal IgE.

Table 5. Haplotype variant frequencies of the PDGFRA gene promoter for different severities as grouped in intermittent and persistent asthma or in 4 grades of asthma (the $2 \times 4 \chi^2$ test was significant: $p = 0.035$)

a Intermittent and persistent asthma

Genotype/allele	Count (frequency)		p value	OR (95% CI)
	intermittent asthma	persistent asthma		
H1	22 (29.7%)	59 (17.9%)		
H2	52 (70.3%)	271 (82.1%)		
H1H1	1 (2.6%)	4 (2%)		
H1H2	20 (52.6%)	51 (30.0%)		
H2H2	16 (42.1%)	110 (64.7%)		
Others	1 (2.6%)	5 (2.9%)		
H1/H2	22/52	59/271	0.021*	1.94 (1.09–3.44)
H1H1 + H1H2/H2H2	21/16	55/110	0.008*	2.625 (1.27–5.43)

b 4 grades of asthma

	Intermittent asthma	Mild persistent asthma	Moderate persistent asthma	Severe persistent asthma	p value	OR (95% CI)
H1H1 + H1H2	21 (27.6%)	20 (26.3%)	24 (31.6%)	11 (14.5%)		
H2H2	16 (12.7%)	34 (27.0%)	44 (34.9%)	32 (25.4%)	0.035*	1.85 (1.12–3.12)

* $p < 0.05$.

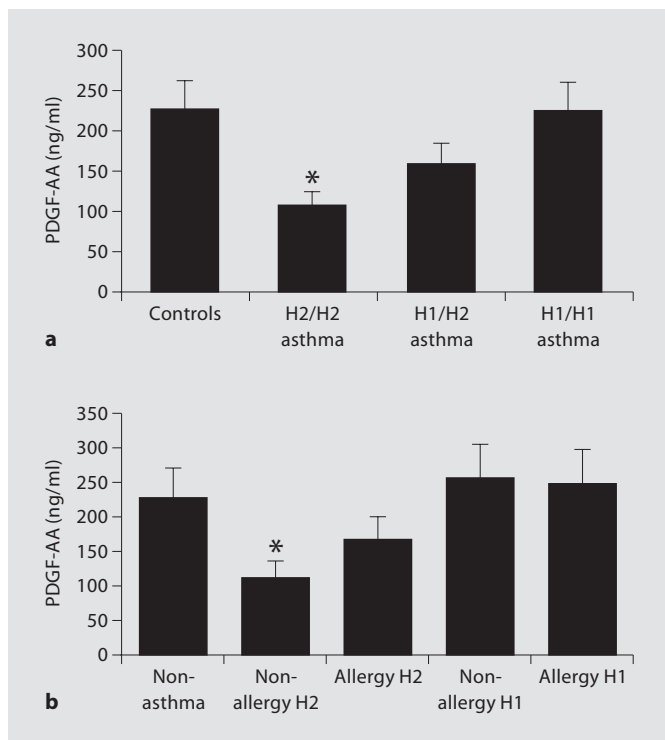


Fig. 2. Serum PDGF-AA levels in different haplotypes of PDGFR α in asthmatic children (a) and in allergic and nonallergic asthmatic children (b). PDGF-AA concentration was measured by the quantitative sandwich enzyme immunoassay technique as described in Materials and Methods. * $p < 0.05$ as compared to nonasthma controls.

frequencies of low (H1) and high (H2) expression of PDGFR α haplotype and alleles were compared in intermittent and persistent asthmatic children, the frequencies of H2 promoter haplotypes as well as the allele of H2/H2 homozygous were more frequent in persistent asthma than in intermittent asthma ($p = 0.021$, OR = 1.94; $p = 0.008$, OR = 2.625, respectively) (table 5a). Moreover, when the severity of asthma was categorized as four grades according to GINA (table 5b), there was also a significantly higher incidence of H2/H2 homozygous haplotypes in severe persistent asthmatic children as compared to intermittent asthma (74.4 vs. 43.2%, $p < 0.001$).

H2H2 Genotype and IgE Level Independently and Additively Contributed to Asthma Severity

We found that asthma severity was related to the patients' allergic status (IgE high or IgE normal) in our study population (table 2). To evaluate the interaction between allergy status and haplotype genotypes, the logistic

regression was performed when the intermittent/persistent status of asthmatic children was used as a dependent variable. The interaction term was not statistically significant ($p = 0.66$). The results of model-fit statistics of logistic regression with two independent dichotomous variables (allergic status and haplotype genotypes) and three independent variables (allergic status, haplotype genotypes, and interaction) were the same (data not shown). These results suggested that the PDGFR α promoter variant independently contributed to asthma severity, and was not related to the patients' allergic status.

The Autocrine Effect of Expression of PDGFR α and Its Ligand, PDGF-AA, in Asthmatic Children

Previously, it was suggested that PDGF-AA, the ligand for PDGFR α , was related to smooth muscle proliferation and fibrosis in vitro. We found that there was no difference of PDGF-AA levels in the sera of normal controls, allergic asthma, and nonallergic asthma, which is in agreement with previous reports [15]. The assessment of the different PDGFR α haplotypes of asthmatic children showed marked decreases of PDGF-AA levels in H2/H2 homozygous haplotype as compared to non-H2/H2 homozygous haplotype asthmatic children (138.1 ± 62.9 vs. 249.7 ± 97.1 ng/ml, $p < 0.05$; fig. 2a), as well as in H2 nonallergic asthmatics as compared to other groups of children (113.8 ± 38.0 vs. 256.6 ± 58.3 ng/ml, $p < 0.05$; fig. 2b). These results reflect that there is an autocrine effect of lower PDGF-AA and higher PDGFR α expression that leads to airway remodeling causing the severity of asthmatic patients.

Discussion

Airway remodeling is the major cause for the chronicity and severity of asthma in adults [13, 16] and children [17]. Much evidence suggests that airway remodeling and peribronchial fibrosis in asthma involves production of extracellular matrix components and collagens by myofibroblasts [18]. PDGF, which is a potent connective tissue mitogen and a competence factor for fibroblast proliferation [9, 19], is reported to play a key role in such fibroblast changes in the lung [20, 21].

In childhood asthma, the atopic status is one of the predisposing and exacerbation factors for the severity of the asthmatic symptoms and the persistence of the disease until late childhood [22, 23]. In our previous study comparing the genetic profiling of allergic and nonaller-

gic asthmatic children, we found that asthmatic children with atopic status and high serum IgE (>1,000 IU) levels were more prone to develop more severe and persistent asthma than nonallergic asthma [10]. Whether this status is related to the progression of airway remodeling is not known. Therefore, this study is the first-ever report to investigate whether the PDGFR α promoter haplotype variants play a significant role in allergic and nonallergic asthmatic children.

In this study, we found there was one of the 5 SNPs, rs1800810 (-1171G/C), tested at the promoter region of the PDGFRA gene that was significantly associated with nonallergic asthma ($p = 0.028$). These SNPs are nearly in complete association. Using haplotype analysis, the combination frequency of low expression of H1 homozygous and heterozygous genotype (H1/H1 + H1/H2) in nonallergic asthmatics was significantly higher in nonallergic asthma as compared to controls (OR = 1.94, CI = 1.11–3.39, $p < 0.02$). H2 promoter haplotypes as well as the allele of H2/H2 homozygous were more frequent in persistent asthma than in intermittent asthma ($p = 0.021$, OR = 1.94; $p = 0.008$, OR = 2.625, respectively). This variant of PDGFR α promoter region contributed independently to asthma severity, and was not related to patients' allergic status. These results suggest that the developmental deficiency due to low expression of PDGFR α may be one of the susceptible factors for nonallergic asthmatic children. In addition, the high expression level of PDGFR α and high serum IgE levels contribute to asthma severity additively, but independently. Our result showed that a lower expression of PDGFR α promoter polymorphism was associated with nonallergic asthma, while the higher expression of PDGFR α promoter polymorphism was associated with the severity of childhood asthma. In the asthmatic children with two copies of high expression variants (H2) there would be a higher chance of asthma becoming persistent and severe. In our view this finding is very important when predicting the long-term outcome for childhood asthma, particularly for early prevention and management.

We also found PDGF-AA, the ligand for PDGFR α , in H2/H2 homozygous haplotype was significantly lower as compared to the non-H2/H2 homozygous haplotype both in allergic asthmatic (138.1 ± 62.9 vs. 249.7 ± 97.1 ng/ml, $p < 0.05$) and nonallergic asthmatic children (113.8 ± 38.0 vs. 256.6 ± 58.3 ng/ml, $p < 0.05$). This result reflected that there was an autocrine effect of lower PDGF-AA and higher PDGFR α expression that might lead to airway remodeling causing the severity of asthma in children.

Although the effect of PDGF on myofibroblasts and tissue fibrotic change is well known in vivo [21] and in vitro [4], the molecular pathogenesis of this inflammatory growth factor and its receptor on allergic airway inflammation and airway remodeling remains obscure. Previously, Aubert et al. [24] reported that the number of PDGF-positive cells and PDGFR β expression were similar among asthmatic subjects, patients with COPD, and nonobstructed smokers. Taylor et al. [25] found no increase in alveolar macrophage PDGF- β mRNA expression in vivo in asthmatic subjects and suggested that PDGF- β chain production by alveolar macrophages is unlikely to play a central role in the pathogenesis of sub-epithelial fibrosis in asthma. This notion is further supported by the finding that levels of PGDF-AA, PGDF-AB, and PGDF-BB are not increased in asthma, and immunohistochemical analysis of PGDF-AA and PGDF-BB and PDGFR α and PDGFR β does not reveal increased expression [26]. In contrast, it is found that eosinophils from asthmatic patients are known to produce the PDGF- β chain [27]. PDGF is also involved in the augmentation of airway responsiveness through remodeling of airway in a mouse model [28]. Moreover, there is strong evidence of activation of PDGF pathway in human asthmatic pulmonary-derived mesenchymal cells [29].

In vivo, the overexpression of IL-1 β in rats via adenoviral transfer increases several profibrotic mediators, including PDGF [30]. IL-1 β is the most potent endogenous factor that induces the PDGFR α on rat lung myofibroblasts in vitro, and PDGFR α up-regulation enhances the mitogenic and chemotactic responses to PDGF isoforms [30, 31]. Induction of the PDGFR α in rat lung myofibroblasts may also be exerted directly by environmental factors, as asbestos and endotoxins have been demonstrated to upregulate this receptor and enhance growth responses to all PDGF isoforms [31, 32]. The feedback and interaction between PDGFR α and its ligands are regulated by IL-1 β and TGF- β 1. Following lung injury, IL-1 β activates p38 MAP kinase, which serves to stabilize the mRNA encoding PDGFR α , thereby increasing cell surface expression of PDGFR α and rendering myofibroblasts hyperresponsive to PDGF-B chain molecules from paracrine sources (e.g., macrophages) or autocrine PDGF-AA. TGF- β 1 serves to downregulate PDGFR α and suppress growth while increasing collagen deposition by myofibroblasts [33]. Our results also revealed that there was an autocrine effect of lower PDGF-AA serum levels and higher PDGFR α expression in H2/H2 homozygous nonallergic asthmatic children, which might lead to airway remodeling causing the severity of asthma.

It is also reported that IL-13, a Th2 cytokine implicated in the pathogenesis of asthma, stimulates the production of PDGF-AA through a STAT-6-dependent mechanism [34]. Recently, Ingram et al. [35] reported that IL-13 acts in synergy with IL-1 β to stimulate myofibroblast growth by coordinately upregulating PDGF-AA and PDGFR α , respectively. These studies emphasize that cytokines elevated early following injury or allergic responses in the lung trigger downstream events that culminate in the proliferation of myofibroblasts through PDGF-dependent mechanisms. It also suggests that inhalation of ambient air pollutants and/or aeroallergens could signal that myofibroblasts become hyperresponsive to PDGF signals, which could contribute sig-

nificantly to airway remodeling in bronchitis and asthma.

In summary, we have observed a statistically significant positive association between polymorphism of PDGFRA promoter region and childhood asthma. The haplotype distribution in our studied subjects implied that the low expression level of the PDGFRA gene was susceptible to nonallergic asthma, while the higher expression level of the PDGFRA gene associated with downregulation of PDGF-AA may contribute to the susceptibility for more severe asthma. Furthermore, this result also implied that chronic asthma initiated by allergy and/or nonallergy factors was determined by different susceptible genetic profiles.

References

- Busse WW, Lemanske RF: Asthma. *N Engl J Med* 2001;344:350–362.
- Cohn L, Elias JA, Chupp G: Asthma: mechanisms of disease persistence and progression. *Annu Rev Immunol* 2004;22:789–815.
- Vignola AM, Costanzo G, Gjomarkaj M, Bonsignore G: Airway remodeling in asthma. *Chest* 2003;123:417S–422S.
- Bonner JC, Osornio-Vargas AR, Badgett A, Brody AR: Differential proliferation of rat lung fibroblasts induced by the platelet-derived growth factor-AA, -AB and -BB isoforms secreted by rat alveolar macrophages. *Am J Respir Cell Mol Biol* 1991;5:539–547.
- Heldin CH, Westermark B: Mechanism of action and in vivo role of platelet-derived growth factor. *Physiol Rev* 1999;79:1283–1316.
- Claesson-Welsh L, Eriksson A, Westermark B, Heldin CH: cDNA cloning and expression of the human A-type platelet-derived growth factor (PDGF) receptor establishes structural similarity to the B-type PDGF receptor. *Proc Natl Acad Sci USA* 1989;86:4917–4921.
- Fruttiger M, Karlsson L, Hall AC, Abramson A, Calver AR, Bostrom H, Willetts K, Bertold CH, Heath JK, Betsholtz C, Richardson WD: Defective oligodendrocyte development and severe hypomyelination in PDGF-A knockout mice. *Development* 1999;126:457–467.
- Joosten PHLJ, Toepoel M, Mariman ECM, Van Zoelen EJJ: Promoter haplotype combinations of platelet-derived growth factor α -receptor gene predispose to human neural tube defects. *Nat Genet* 2001;27:215–217.
- Bonner JC, Badgett A, Lindroos PM, Coin PG: Basic fibroblast growth factor induces expression of the PDGF receptor- α on human bronchial smooth muscle cells. *Am J Physiol* 1996;271:L880–L888.
- Wang JY, Lin G-JC, Bey S-JM, Wang LM, Lin Y-FF, Huang L, Wu S-HL: Discovery of genetic difference between asthmatic children with high IgE level and normal IgE level by whole genome linkage disequilibrium mapping using 763 autosomal STR markers. *J Hum Genet* 2005;50:249–258.
- Spritz RA, Strunk KM, Lee ST, Lu-Kuo JM, Ward DC, Le Paslier D, Altherr MR, Dorman TE, Moir DT: A YAC contig spanning a cluster of human type III receptor protein tyrosine kinase genes (PDGFRA-KIT-KDR) in chromosome segment 4q12. *Genomics* 1994;22:431–436.
- Chen YL, Chen JC, Lin TM, Huang TJ, Wang ST, Lee MF, Wang JY: ABO/secretor genetic complex is associated with the susceptibility of childhood asthma in Taiwan. *Clin Exp Allergy* 2005;35:926–932.
- NHLBI/WHO Workshop Report 2002: Global Strategy for Asthma Management and Prevention. Bethesda, National Institute of Health, National Heart, Lung, and Blood Institute, 2002, Publication No 02-3659.
- Herrmann SM, Ricard S, Nicaud V, Brand E, Behague I, Blanc H, Ruidavets JB, Evans A, Arveiler D, Luc G, Poirier O, Cambien F: Polymorphisms in the genes encoding platelet-derived growth factor A and alpha receptor. *J Mol Med* 2000;78:287–292.
- Baron R, Silverman ES, Grasemann H, Collind T, Drazen JM: DNA sequence variants of the platelet-derived growth factor A-chain gene. *Clin Exp Allergy* 2001;31:1501–1508.
- Lange P, Parner J, Vestbo J, Schnohr P, Jensen G: A 15-year follow-up study of ventilatory function in adults with asthma. *N Engl J Med* 1998;339:1194–1200.
- Payne DN, Rogers AV, Adelroth E, Bandi V, Guntupalli KK, Bush A, Jeffery PK: Early thickening of the reticular basement membrane in children with difficult asthma. *Am J Respir Crit Care Med* 2003;167:78–82.
- Jeffery PK, Godfrey RWA, Adelroth E, Nelson F, Wilson F, Johansson SA: Effects of treatment on airway inflammation and thickening of reticular collagen in asthma: a quantitative light and electron microscopic study. *Am J Respir Crit Care Med* 1995;151:489–494.
- Ross R, Glomset J, Kariya B, Harker L: A platelet-dependent serum factor that stimulates the proliferation of arterial smooth muscle cells in vitro. *Proc Natl Acad Sci USA* 1974;71:1207–1210.
- Bonner JC, Lindroos PM, Rice AB, Moomaw CR, Morgan DL: Induction of PDGF receptor- α in rat myofibroblast during pulmonary fibrogenesis in vivo. *Am J Physiol* 1998;274:L72–L80.
- Hoyle GW, Li J, Finkelstein JB, Eisenberg T, Liu JY, Lasky JA, Athas G, Morris GF, Brody AR: Emphysematous lesion, inflammation, and fibrosis in the lungs of transgenic mice overexpressing platelet derived growth factor. *Am J Pathol* 1999;154:1763–1775.
- Taussig LM, Wright AL, Holberg CJ, Halonen M, Morgan WJ, Martinez FD: Tucson Children's Respiratory Study: 1980 to present. *J Allergy Clin Immunol* 2003;111:661–675.
- Covar RA, Spahn JD, Murphy JR, Szefer SJ: Progression of asthma measured by lung function in the Childhood Asthma Management Program. *Am J Respir Crit Care Med* 2004;170:234–241.

- 24 Aubert JD, Hayashi S, Hards J, Bai TR, Pare PD, Hogg JC: Platelet-derived growth factor and its receptor in lungs from patients with asthma and chronic airflow obstruction. *Am J Physiol* 1994;266:L655-L663.
- 25 Taylor IK, Sorooshian M, Wangoo A, Haynes AR, Kotecha S, Mitchell DM, Shaw RJ: Platelet-derived growth factor-beta mRNA in human alveolar macrophages in vivo in asthma. *Eur Respir J* 1994;11:1966-1972.
- 26 Chanez P, Vignola M, Stenger R, Vic P, Michel FB, Bousquet J: Platelet derived growth factor in asthma. *Allergy* 1995;50:878-883.
- 27 Ohno I, Nitta Y, Yamauchi K, Hoshi H, Honma M, Woolley K, O'Byrne P, Dolovich J, Jordana M, Tamura G: Eosinophils as a potential source of platelet-derived growth factor B-chain (PDGF-B) in nasal polyposis and bronchial asthma. *Am J Respir Cell Mol Biol* 1995;13:639-647.
- 28 Yamashita N, Sekine K, Miyasaka T, Kawashima R, Nakajima Y, Nakano J, Yamamoto T, Horiuchi T, Hirai K, Ohta K: Platelet-derived growth factor is involved in the augmentation of airway responsiveness through remodeling of airways in diesel exhaust particulate-treated mice. *J Allergy Clin Immunol* 2001;107:135-142.
- 29 Malmstrom J, Tufvesson E, Lofdahl CG, Hansson L, Marko-Varga G, Westergren-Thorsson G: Activation of platelet-derived growth factor pathway in human asthmatic pulmonary-derived mesenchymal cells. *Electrophoresis* 2003;24:276-285.
- 30 Lindroos PM, Coin PG, Osornio-Vargas AR, Bonner JC: Interleukin 1 beta (IL-1 beta) and the IL-1 beta-alpha 2-macroglobulin complex upregulate the platelet-derived growth factor alpha-receptor on rat pulmonary fibroblasts. *Am J Respir Cell Mol Biol* 1995;13:455-465.
- 31 Lindroos PM, Coin PG, Badgett A, Morgan DL, Bonner JC: Alveolar macrophages stimulated with titanium dioxide, chrysotile asbestos, and residual oil fly ash upregulate the PDGF receptor-alpha on lung fibroblasts through an IL-1beta-dependent mechanism. *Am J Respir Cell Mol Biol* 1997;16:283-292.
- 32 Coin PG, Lindroos PM, Bird GS, Osornio-Vargas AR, Roggli VL, Bonner JC: Lipopolysaccharide up-regulates platelet-derived growth factor (PDGF) alpha-receptor expression in rat lung myofibroblasts and enhances response to all PDGF isoforms. *J Immunol* 1996;156:4797-4806.
- 33 Bonner JC: Regulation of PDGF and its receptors in fibrotic diseases. *Cytokine Growth Factor Rev* 2004;15:255-273.
- 34 Ingram JL, Rice A, Geisenhoffer K, Madtes DK, Bonner JC: Interleukin-13 stimulates the proliferation of lung myofibroblasts via a signal transducer and activator of transcription-6-dependent mechanism: a possible mechanism for the development of airway fibrosis in asthma. *Chest* 2003;123:422S-424S.
- 35 Ingram JL, Rice AB, Geisenhoffer K, Madtes DK, Bonner JC: IL-13 and IL-1beta promote lung fibroblast growth through coordinated up-regulation of PDGF-AA and PDGF-R alpha. *FASEB J* 2004;18:1132-1134.