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Association of peroxisome proliferator-activated receptor-gamma gene polymorphisms with the development of asthma

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Summary
Background: The peroxisome proliferator-activated receptors (PPAR) are the nuclear hormone receptor superfamily of ligand-activated transcriptional factors. PPAR-gamma (PPARG) activation downregulates production of Th2 type cytokines and *eosinophil* function. Additionally, treatment with a synthetic PPARG ligand can reduce lung inflammation and IFN-gamma, IL-4, and IL-2 production in experimental allergic asthma. In patients with asthma, *PPARG* gene expression is known to be associated with the airway inflammatory and remodeling responses. Thus, genetic variants of *PPARG* may be associated with the development of asthma.
Methods: We genotyped two single nucleotide polymorphisms on the *PPARG* gene, *+34C > G* (Pro12Ala) and *+82466C > T* (His449His), in Korean subjects (839 subjects with asthma and 449 normal controls).
Results: Association analysis using logistic regression analysis showed that *+82466C > T* and haplotypes 1(CC) and 2(CT) were associated with the development of asthma ($p = 0.01$–0.04). The frequency of *PPARG-ht2* was significantly lower in the patients with asthma compared to the normal controls in codominant and dominant models ($p = 0.01$, $p_{corr} = 0.03$ and $p = 0.02$, $p_{corr} = 0.03$, respectively). Conversely, the frequency of *PPARG-ht1* was significantly higher in

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the patients with asthma compared to the normal controls in the codominant model [$p = 0.04$, OR: 1.27 (1.01–1.6)]. In addition, the rare allele frequency of $+82466C > T$ was significantly lower in patients with asthma in comparison to normal controls in the codominant model (OR: 0.78, $p = 0.04$). Thus, polymorphism of the *PPARG* gene may be linked to an increased risk of asthma development.
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Introduction

The peroxisome proliferator-activated receptors (PPAR) are the nuclear hormone receptor superfamily of ligand-activated transcriptional factors, which include receptors for steroids, thyroid hormone, vitamin D, and retinoic acid.[1] Three subtypes of PPAR are known, PPAR-alpha, PPAR-delta, and PPAR-gamma (PPARG). *PPARG* (MIM# 601487), located on chromosome 3p25, was originally characterized as a regulator of adipocyte differentiation and lipid metabolism,[2] and of cellular turnover.[3] In addition, PPARG activation has been known to downregulate the synthesis and release of immune modulating cytokines from various cell types.[4] That a range of naturally occurring substances, including the metabolites of arachidonate pathway, such as 15-hydroxyeicosatetranoic acid (15-HETE), or Th2 cytokines, such as IL-4, are potent inducers of *PPARG* expression has been well established.[5] In contrast, stimulation of the PPARG ligand was found to significantly inhibit production of the Th2 type cytokines and downregulate eosinophil functions.[6,7] Additionally, treatment with a synthetic PPARG ligand can reduce lung inflammation and IFN-gamma, IL-4, and IL-2 production in experimental allergic asthma.[8] In subjects with asthma, *PPARG* expression is known to be associated with the airway inflammatory and remodeling responses.[9] Thus, genetic variants of the *PPARG* gene may be associated with the development of asthma.

Recently, Palmer et al. reported that *PPARG* gene polymorphisms were associated with the risk of asthma exacerbation in Caucasian populations.[10] The homozygous haplotype combination of $+34C > G$ (Pro12Ala) was associated with an increased risk for asthma exacerbation.[10] However, to the best of our knowledge, no previous study has analyzed potential associations between the two common polymorphisms of the *PPARG* gene, $+34C > G$ (Pro12Ala) and $+82466C > T$ (His449His), with the risk of asthma.

Materials and methods

Subjects

The subjects were recruited from the Asthma Genome Research Center, which consists of Soonchunhyang University hospitals in Bucheon, Seoul, and Chunan, Korea. All of the subjects were Korean. A clinical history was obtained for each patient using a physician-administered questionnaire.[11] And the patients with asthma had compatible clinical symptoms and physical characteristics.[12] Each patient showed airway reversibility [as documented by inhalant bronchodilator-induced improvement of more than 15% of forced expiratory volume in 1 s (FEV_1)] and/or airway hyperreactivity of less than 10 mg/ml of methacholine. Normal controls ($n = 449$) were recruited from spouses of the patients or members of the general population who answered negatively to a screening questionnaire regarding respiratory symptoms.[11] The controls had FEV_1 values > 80% predicted, PC_{20} methacholine > 10 mg/ml, and normal findings on simple chest radiograms. Skin prick tests were performed with 24 common aeroallergens.[13] Atopy was defined as one or more positive reactions (>3 mm in diameter or greater than histamine reaction of 1 mg/ml) on the skin prick test. Total IgE was measured using the UniCAP system (Pharmacia Diagnostics, Uppsala, Sweden). The subjects with diabetes mellitus were excluded because the *PPARG* polymorphism was reported to be associated with the development of diabetes mellitus in Korea.[14] All subjects gave written informed consent to participate in the study, and the protocols were approved by the local ethics committees.

Genotyping of the single nucleotide polymorphism (SNPs) on the *PPARG* gene

For genotyping of polymorphic sites, amplifying primers and probes were designed for TaqMan® (Table 1) and the single

Table 1 Primer sequence for genotyping of SNPs on *PPARG* gene.

Approaches	Loci	Region	Primer sequence	Orientation	Location
Variant Screening					
	$+34C > G$	Exon3	GTTATGGGTGAAACTCTGGGAGATT	Forward	−3/+22
			GCAGACAGTGTATCAGTGAAGGAAT	Reverse	+44/+68
			ATTGACCCAGAAAG	VIC	+23/+41
			ATTGACGCAGAAAG	FAM	
	$+82466C > T$	Exon8	CAGAAAATGACAGACCTCAGACAGA	Forward	+824271/+82442
			CGTCTTCTTGATCACCTGCAGTAG	Reverse	+82463/+82486
			CTGCACGTGTTCCG	VIC	+82449/+82462
			CTGCACATGTTCCG	FAM	

Table 2 The clinical profiles of the study subjects.

	Normal controls	Asthmatics	p-Value
Number	449	839	–
Age [year (range)]	44 (5–80)	46 (8–80)	0.369
Duration [year (range)]	–	4 (1–70)	–
On set of age [year (range)]	–	38 (1–76)	–
Sex (male,%)	40.31	39.57	0.796
Smoker/ex-smoker (%)	17.16/10.53	16.21/16.21	0.022
Atopy (%)	35.15	60.22	<0.0001
FVC (% predicted)	94.24 ± 0.59	83.51 ± 0.62	<0.0001
FEV_1 (% predicted)	104.14 ± 0.72	79.63 ± 0.76	<0.0001
log [PC20 methacholine] (mg/ml)	1.39 ± 0.002	0.17 ± 0.02	<0.0001
BMI (kg/m^2)	23.81 ± 0.16	24.19 ± 0.12	0.073
Blood eosinophil (%)	2.37 ± 0.1	5.61 ± 0.19	<0.0001
log [total IgE] (IU/ml)	1.66 ± 0.03	2.17 ± 0.02	<0.0001

Values are mean ± S.E.
P-values are obtained using *t*-test or χ^2 test between asthmatics and normal controls.

base extension method. Primer Express (Applied Biosystems, Foster City, CA, 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. Typically, PCR was run in the TaqMan Universal Master mixture without UNG (Applied Biosystems) at a primer concentration of 900 nM and TaqMan MGB-probe concentration of 200 nM. The reaction was performed in a 384-well format in a total reaction volume of 5 μl using 20 ng of genomic DNA. The plate was then placed in a thermal cycler (PE 9700; Applied Biosystems) and heated for 2 min at 50 °C and for 10 min at 95 °C, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. The TaqMan assay plate was then transferred to a Prism 7900 HT instrument (Applied Biosystems), where the fluorescence intensity of each well was read. Fluorescence data files from each plate were analyzed by automated software (SDS 2.1).

Statistics

We applied the widely used Lewontin's D′ (|D′|) and R^2 measures of linkage disequilibrium to all pairs of biallelic loci.[15] Haplotype associations were estimated using HaploScore (http://www.biostat.wustl.edu/genetics/geneticssoft/), which computes score statistics to test for associations between a given haplotype and a wide variety of traits, including binary, ordinal, quantitative, and Poisson. The genetic effects of the haplotypes were analyzed in the same way as the SNPs. The distributions of the *PPARG* SNP genotypes and haplotypes among the subjects with asthma and the normal subjects were analyzed with logistic regression models that controlled for age (continuous value), sex (male = 0, female = 1), atopy status (non-atopy = 0, atopy = 1), body mass index (BMI; continuous value) and smoking status (non-smoker = 0, ex-smoker = 1, smoker = 2) as covariables. The data were managed and analyzed using SPSS ver. 10.0 (SPSS Inc., Chicago, IL, USA) software packages. A $p \leq 0.05$ was regarded as statistically significant. For correction of multiple testing, the effective number of independent markers in *PPARG* was calculated using the software SNPSpD (http://genepi.qimr.edu.au/general/daleN/SNPSpD), which is based on the spectral decomposition (SpD) of matrices of pairwise linkage disequilibrium (LD) between SNPs.[16] The number of independent marker loci in *PPARG* was calculated as 1.8712, and this was applied to correct for multiple testing (p-value × 1.8712). Statistical powers were calculated using the Statistical Power Calculator (http://www.dssresearch.com/toolkit/spcalc/power_p2.asp). A two-tailed test was used on allele frequencies for both case and control subjects at a 5% alpha level.

Results

Characteristics of the study subjects

Significant differences were observed in the smoking status and prevalence of atopy between the controls and subjects with asthma (Table 2). The FEV_1% and PC_{20} methacholine values of the patients with asthma were significantly lower than those of the normal controls, whereas the total IgE level was significantly higher in the former compared to the latter.

Table 3 The frequencies, heterozygosity and Hardy–Weinberg Equation of SNPs on *PPARG* gene in the study population.

Gene	Loci	rs SNP	Region	A.A change	Genotype				Frequency	Heterozygosity	HWE
					C/C	C/R	R/R	*N*			
PPARG	*+34C > G*	rs 1801282	Exon3	Pro12Ala	1180	104	4	1288	0.043	0.083	0.294
	+82466C > T	rs 3856806	Exon8	His449His	894	358	36	1288	0.167	0.278	0.982

C/C, C/R and C/C represent common allele, heterozygosity and rare allele.
**p*-Values of deviation from Hardy–Weinberg Equilibrium in the study population.

Association of SNPs of the *PPAR* gene with the risk of asthma

Two SNPs on the *PPARG* gene were genotyped for the association study: $+34C>G$ (P12A) on exon 3 and $+82466C>T$ (H449H) on exon 8. The minor allele frequencies (MAF) of these two SNPs in the Korean population were 0.043 ($+34C>G$) and 0.167 ($+82466C>T$) (Table 3). The genotype distributions of the loci were in Hardy–Weinberg equilibrium ($p>0.05$; Table 3). Four haplotypes were constructed, and two haplotypes with a frequency >0.05 were used for the analysis. The $+82466C>T$ SNP was found to be associated with asthma development. The rare allele frequency was significantly lower in patients with asthma compared to that of the normal controls in a codominant model (OR: 0.78, $p=0.04$). Haplotypes of *PPARG* were also associated with the development of asthma (Table 4). The frequency of *PPARG-ht2* was significantly lower in the subjects with asthma compared to the normal controls in both codominant and dominant models [$p=0.01$, $p_{corr}=0.03$, OR: 0.73 (0.57–0.94) and $p=0.02$, $p_{corr}=0.03$, OR: 0.71 (0.53–0.94), respectively]. Conversely, the frequency of *PPARG-ht1* was significantly higher in those with asthma compared to the normal controls in a codominant model [$p=0.04$, $p_{corr}=0.08$, OR: 1.27 (1.01–1.6)].

Discussion

In the present study, we are the first to show that *PPARG* polymorphisms are associated with the risk of developing asthma. The frequency of *PPARG-ht2* was significantly lower, and the frequency of *PPARG-ht1* was higher in the subjects with asthma compared to the normal controls. These data suggest that haplotype-2 may protect against the development of asthma while haplotype-1 may be linked to asthma predisposition. Recently, Palmer et al. reported that two SNPs of *PPARG* are associated with the risk of asthma exacerbation in Caucasian populations.[10] Moreover, they observed no difference in allele frequency from the normal controls. Thus, we tried to evaluate the association of the two SNPs ($+34C>G$ and $+82466C>T$) with asthma development. However, since we did not examine the exacerbation rate in our asthma cohort, we were unable to replicate the link between asthma exacerbation rate and SNPs as demonstrated by Palmer et al.[10]

Because of the biological properties of PPARG as a regulator of adipocyte differentiation and lipid metabolism,[2] polymorphisms of the *PPARG* gene have been associated with diabetes, obesity, and various metabolic syndromes.[17] The two common polymorphisms of *PPARG* were reported to modify susceptibility to type 2 diabetes mellitus, obesity, and subtypes of metabolic syndrome in Korean[14,17] and Caucasian populations.[18,19] In accordance with these findings, we excluded the subject with diabetes from our analysis and adjusted the association analysis with BMI.

In addition to the metabolic effect, PPAR form heterodimers with retinoid X receptors (RXR) and these heterodimers regulate transcription of various genes that participate in allergic inflammation and airway remodeling.[9,20] In addition to regulating inflammation and

Table 4 Genotype and haplotype distribution of *PPARG* polymorphisms in asthmatics and normal controls of the study subjects.

Loci	Position	rs#	Genotype	N (%)		Co-dominant			Dominant			Recessive		
				BA	NC	OR (95%CI)	p	p_{corr}	OR (95%CI)	p	p_{corr}	OR (95%CI)	p	p_{corr}
$+34C>G$	Exon3	rs1801282	C	770 (91.78%)	410 (91.31%)	1.07 (0.69–1.65)	0.77	1.54	1.12 (0.7–1.77)	0.64	1.29	0.49 (0.07–3.74)	0.5	0.99
			CG	67 (7.99%)	37 (8.24%)									
			G	2 (0.24%)	2 (0.45%)									
$+82466C>T$	Exon8	rs3856806	C	596 (71.04%)	298 (66.37%)	0.78 (0.62–0.98)	*0.04*	0.07	0.78 (0.6–1.02)	0.07	0.15	0.54 (0.26–1.09)	0.09	0.17
			CT	224 (26.7%)	134 (29.84%)									
			T	19 (2.26%)	17 (3.79%)									
ht1(CC)	–	–	−/−	20 (2.38%)	18 (4.01%)	1.27 (1.01–1.6)	*0.04*	0.08	1.87 (0.93–3.73)	0.08	0.16	1.26 (0.97–1.64)	0.09	0.17
			ht1/−	235 (28.01%)	139 (30.96%)									
			ht1/ht1	584 (69.61%)	292 (65.03%)									
ht2(CT)	–	–	−/−	648 (77.23%)	325 (72.38%)	0.73 (0.57–0.94)	*0.01*	*0.03*	0.71 (0.53–0.94)	*0.02*	*0.03*	0.61 (0.25–1.44)	0.26	0.51
			ht2/−	178 (21.22%)	114 (25.39%)									
			ht2/ht2	13 (1.55%)	10 (2.23%)									

NC and BA represent normal controls and asthmatics, respectively. C/C, C/R and C/C represent common allele, heterozygosity and rare allele.
The p-values were obtained by logistic regression analysis, controlled for age (continuous value), sex (male = 0, female = 1), atopy status (non-atopy = 0, atopy = 1), and smoking status (non-smoker = 0, ex-smoker = 1, smoker = 2) as covariables.
Italic faces mean the $p<0.05$.

immunity,[21] PPARG also plays important roles in controlling cell differentiation and fibrotic responses of the lung.[22] In comparison to normal subjects, subjects with asthma who were not treated with steroids have increased expressions of *PPARG*, particularly in the airway epithelium and smooth muscle.[9] PPARG is involved in a cross talk with glucocorticoid receptors or beta-2 adrenergic receptors.[23] *PPARG*, together with its synthetic agonists, appears to play an important role in inflammation, and recent evidence indicates that its expression is augmented in association with features of early airway remodeling in patients with asthma.[9] Thus, $PPARG + 82466C > T$ may contribute to the development of asthma via functional changes in the gene.

In summary, we genotyped two SNPs on *PPARG*, $+34C > G$ (Pro12Ala) and $+82466C > T$ (His449His) and examined their link to asthma development. Association analysis showed that $PPARG + 82466C > T$ and *PPARG*-haplotypes were associated with the development of asthma. Thus, the $+34C > G$ and $+82466C > T$ polymorphisms present in the coding region of *PPARG* may influence asthma predisposition via regulation of gene expression. This information may contribute to the development of new strategies for the diagnosis and control of asthma.

Conflict of interest statement

None of the authors have a conflict of interest to declare regarding this study.

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