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Genetic polymorphisms at *FCER1B* and *PAI-1* and asthma susceptibility

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Clinical and
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Summary

Background We previously detected a promoter polymorphism (– 109C/T) in the gene for the β -chain of the high-affinity receptor for IgE (*FCER1B*), which was associated with total serum IgE levels but not with asthma in a Japanese population. A genetic interaction is biologically plausible between Fc ϵ RI- β and the plasminogen activator inhibitor 1 (PAI-1), which is highly expressed in mast cells in asthmatics and plays an essential role in airway remodelling. We hypothesized that *FCER1B* promoter polymorphisms, by modifying the intensity of mast cell activation signals, modulate the genetic effects of a functional 4G/5G polymorphism in the *PAI-1* gene on asthma.

Objective To examine whether *FCER1B* promoter polymorphisms (– 109C/T and – 654C/T) influence the genetic effects of the functional polymorphism (4G/5G) at the *PAI-1* promoter region on asthma susceptibility using a case-control analysis.

Methods Subjects (374 asthmatic patients and 374 non-asthmatic controls) were divided into combined genotype groups based on the presence of *FCER1B* – 109TT and – 654CC genotypes and the *PAI-1* 4G allele. Logistic regression analysis was used to estimate adjusted odds ratios for asthma associated with the different genotype groups.

Results Individuals homozygous for the *FCER1B* – 109T/– 654C haplotype and the *PAI-1* 5G allele had a reduced susceptibility to asthma; the odds ratio for the development of asthma was 0.20 (95% confidence interval, 0.084–0.46; $P = 0.00015$) for them, compared with individuals also homozygous for the – 109T/– 654C haplotype at *FCER1B* but carrying the 4G allele at *PAI-1*. The regression model also showed an interaction of the *PAI-1* 4G/5G genotype with the *FCER1B* – 109C/T (P for interaction = 0.0017) or *FCER1B* – 654C/T (P for interaction = 0.031) on asthma.

Conclusion The present findings suggest a synergistic interaction between *FCER1B* and *PAI-1* genes in asthma susceptibility.

Keywords bronchial asthma, Fc ϵ RI- β , gene-gene interaction, PAI-1

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Introduction

The plasminogen activator system plays an important role in control of endogenous fibrosis and regulation of extracellular matrix (ECM) proteolysis relevant to tissue remodelling [1]. Comprehensive microarray analysis of human gene expression profiles has shown that the *PAI-1* gene is expressed at its highest level in activated mast cells following IgE receptor cross-linking [2]. A murine asthma model has shown that plasminogen activator inhibitor 1 (*PAI-1*)-dependent inhibition of matrix metalloproteinase (MMP)-9 activity and fibrinolysis contributes to ECM deposition in airways [3]. The *PAI-1* gene has a 4G/5G

variation in the promoter region, and the 4G allele correlates with increased basal transcription of *PAI-1* and higher activity of plasma PAI-1 [4]. Results of the Nottingham Asthma Family study [5] and a study of Czech subjects [6] suggested that the presence of the 4G allele is associated with asthma.

The high-affinity receptor for IgE (Fc ϵ RI) plays a central role in the induction and maintenance of an allergic response by mast cells and basophils. The β -chain of the Fc ϵ RI plays a critical role in setting the level of cellular response to IgE and antigen through its capacity to amplify Fc ϵ RI signalling and cell-surface expression. Polymorphisms at the gene encoding the β -chain of Fc ϵ RI

(*FCER1B*) have been repeatedly associated with elevated total and specific IgE levels [7, 8]. In a previous study with Japanese subjects, we detected a single-nucleotide polymorphism (–109C/T) in the promoter region of the *FCER1B* gene, and found a significant association of the –109 TT genotype with higher levels of total serum IgE in asthmatic patients, although this polymorphism was not associated with asthma [9]. The *FCER1B* promoter haplotype that includes the –109T and –654C alleles has been recently associated with decreased levels of FcεRI on the cell surface of peripheral blood basophils, and with lower transcriptional activity *in vitro* [10]. However, there is a strong linkage disequilibrium between each of 26 polymorphisms from –654 to +14050 bp in the genomic sequence of *FCER1B* and upstream and downstream DNA [11], so the promoter haplotype consisting of –109T and –654C alleles may be a neutral marker in tight linkage to a true functional variant in this region.

It is likely that several genes, each with minor-to-moderate effects, act together with environmental factors to determine the overall risk of asthma. Evidence is beginning to emerge in the literature suggesting that epistatic interactions among genes are probably more common than previously thought for asthma [12, 13]. Considering the biological plausibility of interactions between FcεRI-β and PAI-1, the specific aim of the current study is to examine whether the *FCER1B* promoter polymorphism, which modifies the intensity of cell activation signals in mast cells, also moderates the genetic effects of the *PAI-1* promoter polymorphism on asthma.

Methods

Study population

The present case-control study included 748 unrelated Japanese subjects, mostly from the Sapporo area of Hokkaido, Japan. Patients with physician-diagnosed asthma ($n = 374$; 165 males and 209 females) were recruited from the pulmonary clinic at the First Department of Medicine, Hokkaido University Hospital. Asthma was defined on the basis of recurrent episodes of at least two of three symptoms (cough, wheeze, or dyspnoea) that were associated with a demonstrable reversible airflow limitation and/or increased airway responsiveness to methacholine, as described previously [14, 15]. A total of 374 control subjects (246 males and 128 females) with no history of bronchial asthma or other respiratory symptoms were selected from among healthy volunteers who visited our clinic for routine annual physical examinations. All participants underwent a structured interview and physical examination. We recorded the age, sex, smoking status (currently, formerly, or never), atopic status (positive specific IgE responses to at least one of

10 common inhaled allergens), and total serum IgE levels of every participant.

We obtained approval from the relevant ethics committees, and obtained written informed consent of participants.

Genotyping

FCER1B –109C/T, –654C/T, and *PAI-1* 4G/5G promoter polymorphisms were genotyped using genomic DNA and an assay that combines kinetic (real-time quantitative) PCR with allele-specific amplification, as described previously [14, 15]. At *FCER1B* –109C/T and *PAI-1* 4G/5G polymorphisms, complete genotypes were obtained for 374 asthmatic patients and 374 healthy controls, including 339 asthmatics and 305 healthy controls whom we previously genotyped for the –109C/T promoter polymorphism at *FCER1B* [16]. For 10 individuals (three asthmatics and seven controls), genomic DNA failed to amplify and genotypes were missing at the *FCER1B* –654C/T polymorphism.

Statistical analyses

Hardy-Weinberg equilibrium was tested using the χ^2 test. Statistical analyses were based on the calculation of odds ratios to provide estimates of the relative risk of bronchial asthma. To evaluate the interaction of the *PAI-1* 4G/5G promoter polymorphisms with *FCER1B* –109C/T and/or –654C/T polymorphisms, we divided subjects into four or eight combined genotype groups based on the presence of a *PAI-1* 4G allele and homozygosity for *FCER1B* –109T and/or –654C alleles, because previous studies showed that carrying the –109TT genotype and the 4G allele was associated with increased levels of total serum IgE [9] and asthma [5, 6], respectively. Logistic regression analysis was used to estimate adjusted odds ratios for asthma associated with the different genotype groups; the reference group was individuals who carry both the –109TT and –654CC genotype at *FCER1B* and the 4G allele at *PAI-1*. We also examined the interaction of two-gene genotypes by entering an interaction term of '*FCER1B* × *PAI-1*' into the regression models. All of the regression analyses were adjusted for sex, age, smoking status (currently, formerly, or never), and total serum IgE levels as potential confounding variables.

Results

The median age of the asthmatics was 45 years (range 16–81), and the median age of the healthy controls was 32 years (18–72) (Table 1). We found that 71.9% of the asthmatics and 56.1% of the controls were atopic. The total serum IgE level was much higher in asthmatics (mean log (total IgE), 2.40 log IU/mL; standard deviation

Table 1. Basic characteristics and genotype frequencies in 374 patients with asthma and in 374 controls

	Patients with asthma	Controls
Number of subjects	374	374
Age (median (range))	45 (16–81)	32 (18–72)*
Sex (male/female)	246/128*	165/209
Current smoker (%)	21.70	27.0*
Total IgE (log IU/mL) (SD)	2.40 (0.64)	1.86 (0.64)*
Atopy [†] (%)	71.90	56.1*
Age at onset (median (range))	33 (0–77)	–
<i>FcεRIβ</i> genotypes ^{‡§}		
– 109T/T	157	156
– 109T/C	178	169
– 109C/C	39	49
– 654C/C	262	267
– 654C/T	99	98
– 654T/T	6	6
<i>PAI-1</i> genotype [‡]		
4G/4G	131	130
4G/5G	194	185
5G/5G	49	59

**P* value < 0.0001 for differences between patients with asthma and controls.

[†]Atopy is defined as the presence of specific IgE antibody to at least one of 10 common inhaled allergens.

[‡]None of the polymorphisms were associated with asthma (by $2 \times 3 \chi^2$ analyses).

[§]We failed to detect the – 654C/T genotype in 10 subjects (seven asthmatics and three controls).

(SD) 0.67) than in controls (mean, 1.86 log IU/mL; SD 0.61).

At three promoter polymorphisms at *FCER1B* and *PAI-1*, no deviation from the Hardy–Weinberg equilibrium was observed among the 374 asthmatics or among the 374 controls. The – 109C/T and – 654C/T polymorphisms were in a significant linkage disequilibrium, with the – 109T allele being strongly associated with the – 654C allele ($P < 0.000001$). Although none of these three polymorphisms was associated with asthma (Table 1), a synergistic effect was observed when we stratified the study sample according to the presence of *PAI-1* 4G allele and *FCER1B* – 109TT and/or – 654CC genotype. In individuals who had the – 109TT or the – 654CC genotype at *FCER1B*, the presence of a *PAI-1* 4G allele was significantly associated with asthma (Table 2). In addition, an interaction was found between the *FCER1B* promoter haplotype and *PAI-1* 4G/5G polymorphism; among individuals who were homozygous for the – 109T/– 654C haplotype, the odds ratio was 0.20 (95% confidence interval (CI), 0.084–0.46; $P = 0.00015$) for individuals who were also homozygous for a *PAI-1* 5G allele compared with those carrying a *PAI-1* 4G allele (Table 2). The interaction of the *PAI-1* 4G/5G polymorphism with *FCER1B* – 109T/C and – 654C/T polymorphisms was

also demonstrated in the regression analysis by formulating product variables of pairs of genotypes in the model (P for interaction between *FCER1B* – 109C/T and *PAI-1* 4G/5G = 0.0017, and P for interaction between *FCER1B* – 654C/T and *PAI-1* 4G/5G = 0.031).

Discussion

Asthma is a complex disease, and interactions among numerous genetic and environmental factors likely play an important role in its pathogenesis. Although our finding is consistent with the previous studies that showed an association of the *PAI-1* 4G allele with the development of asthma [5, 6], the genetic impact of the 4G allele was evident only in carriers of particular *FCER1B* genotypes, which represents a possible genetic interaction between two or three common alleles at polymorphic loci. Neither *FCER1B* nor *PAI-1* polymorphisms were individually associated with asthma, which indicates that the two genes have a synergistic interaction rather than an additive effect in the pathogenesis of asthma. Recently, a possible promoter-dependent functional mechanism has been reported for a genetic association between *FCER1B* and atopy [10]; a common haplotype consisting of – 109T, – 426T, – 654C, and – 752T in the *FCER1B* promoter region has been associated with decreased cell-surface expression of FcεRI on peripheral blood basophils, and with a lower promoter activity *in vitro*. Therefore, our findings suggest that individuals who were homozygous for *FCER1B* – 109T/– 654C haplotype and for the *PAI-1* 5G allele, both of which are associated with lower promoter activity of the respective genes, were less susceptible to the development of asthma.

A murine model of allergic airway hyper-reactivity suggests that disordered coagulation and fibrinolysis may contribute to the pathogenesis of asthma [17]. *In vitro*, the fibrinolytic and coagulation systems can be activated or inactivated by mast cell and basophil mediators [18]; mast cells and basophils, by expressing PAI-1 when activated through the FcεRI-β, participate in the modulation of the balance between proteases and protease inhibitors that regulates tissue injury and repair in asthma [2]. Our finding suggests that *FCER1B* and *PAI-1* may be involved in the same biological pathways of the remodeling process in asthmatic airways, and homozygosity for the *FCER1B* – 109T/– 654C haplotype and the *PAI-1* 5G allele may critically alter the balance between plasmin and plasmin inhibitor, which consequently reduces susceptibility to airway remodelling [1]. There may, however, be multiple different pathways underlying our observation of a genetic interaction between *FCER1B* and *PAI-1* genotype, and on the basis of the present results, it is not possible to explain the underlying mechanism fully. Further studies will be necessary to provide the functional relevance of our findings.

Table 2. Impact of the PAI-1 4G/5G genotype on asthma according to the FCER1B – 109C/T and – 654C/T genotype

<i>PAI-1</i> genotype	<i>FCER1B</i> genotype (– 109)		<i>N</i> (asthma/control)	OR*	95% CI	<i>P</i>
4G/4G or 4G/5G	TT		142/126	1	Reference	
5G/5G	TT		15/30	0.213	0.09, 0.48	0.00023
4G/4G or 4G/5G	CT or CC		183/189	0.999	0.68, 1.46	0.99
5G/5G	CT or CC		34/29	1.128	0.58, 2.18	0.72
<i>PAI-1</i> genotype	<i>FCER1B</i> genotype (– 654)		<i>N</i> (asthma/control)	OR*	95% CI	<i>P</i>
4G/4G or 4G/5G	CC		233/220	1	Reference	
5G/5G	CC		29/47	0.38	0.55, 3.13	0.0019
4G/4G or 4G/5G	CT or TT		87/92	0.93	0.21, 0.70	0.76
5G/5G	CT or TT		18/12	1.32	0.62, 1.41	0.55
<i>PAI-1</i> genotype	<i>FCER1B</i> genotype (– 109)	<i>FCER1B</i> genotype (– 654)	<i>N</i> (asthma/control)	OR*	95% CI	<i>P</i>
4G/4G or 4G/5G	TT	CC	137/121	1	Reference	
4G/4G or 4G/5G	TT	CT or TT	2/4	– [†]	–	–
4G/4G or 4G/5G	CT or TT	CC	96/99	1.03	0.66, 1.61	0.9
4G/4G or 4G/5G	CT or TT	CT or TT	85/88	0.97	0.61, 1.54	0.9
5G/5G	TT	CC	14/30	0.20	0.084, 0.46	0.00015
5G/5G	TT	CT or TT	1/0	– [†]	–	–
5G/5G	CT or TT	CC	15/17	0.88	0.36, 2.13	0.78
5G/5G	CT or TT	CT or TT	17/12	1.29	0.52, 3.23	0.58

*ORs were adjusted for age, sex, smoking status, and total IgE levels using a multivariate logistic regression model.

[†]Because the number of subjects was small, ORs were not calculated for these subgroups.

OR, odds ratio; CI, confidence interval.

Gene–gene interaction between *FCER1B* and *PAI-1* for asthma susceptibility remained significant after Bonferroni's correction for the multiple comparisons in the study. The biological understandings of the pathogenesis of asthma indicate that both FcεRI-β and *PAI-1* are significantly involved in asthma. Furthermore, we have studied three genetic polymorphisms at *FCER1B* and *PAI-1*, all of which have some relevance to functional alternations and have also been associated with asthma and atopic phenotypes in previous genetic studies [5–9]. The interaction between *FCER1B* and *PAI-1* genes is also biologically plausible; in general, risk interactions are more plausible between genes involved in a physical interaction found in the same biological pathways or involved in the same regulatory network. Collectively, we believe that our findings of a significant association are causal rather than a result of chance alone. Nevertheless, this study should be considered as exploratory, generating hypotheses rather than testing hypotheses, and the present evidence for genetic interaction requires confirmation using large independent datasets, especially because the frequency of individuals homozygous for the *PAI-1* 5G allele, which is involved in the positive findings in the current study, is rather small.

In conclusion, we used a functional polymorphism in the promoter region of the *PAI-1* gene to characterize genetic vulnerability to airway remodelling, and found that homozygotes for *PAI-1* 5G allele, which is associated with lower PAI-1 activity, have a decreased risk of asthma if they are also homozygous for the *FCER1B* – 109T and

– 654C alleles. Our findings identify a group at decreased risk for asthma, and identification of genetic factors of asthma and their interactions, such as the interaction between *PAI-1* and *FCER1B* variants, can provide a basis for a better understanding of the pathogenic molecular mechanisms underlying asthma. These findings, if confirmed, could also have clinical implications for the prevention and treatment of bronchial asthma.

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