

IL-17F sequence variant (His161Arg) is associated with protection against asthma and antagonizes wild-type IL-17F activity

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Background: IL-17F is a recently discovered cytokine that plays a role in tissue inflammation by inducing release of proinflammatory and neutrophil-mobilizing cytokines. Upregulated *IL17F* gene expression has been observed at sites of allergen challenge in the airways of patients with asthma, suggesting that IL-17F is involved in the pathophysiology of asthma.

Objective: To investigate the role of IL-17F in asthma pathogenesis, we conducted genetic analyses of association of asthma with the common variants of *IL17F*, using 867 unrelated Japanese subjects.

Methods: Five polymorphisms were studied, including the coding-region sequence variant single nucleotide polymorphism rs763780 (7488T/C), which causes a His-to-Arg substitution at amino acid 161 (H161R). Functional consequences of the H161R substitution were examined by using recombinant wild-type and mutant IL-17F proteins.

Results: Homozygosity of the H161R variant was inversely associated with asthma; the odds ratio (95% CI) for asthma was 0.06 (0.01-0.43) for the H161R homozygote compared with the wild-type homozygote ($P = .0039$). This result remains significant ($P = .0079$) after adjustment for the presence of atopy using the Mantel-Haenszel χ^2 test. In addition, *in vitro* functional experiments demonstrated that the H161R variant of IL-17F lacks the ability to activate the mitogen-activated protein kinase pathway, cytokine production, and chemokine production in bronchial epithelial cells, unlike wild-type IL-17F. Furthermore, the H161R variant blocked induction of IL-8 expression by wild-type IL-17F.

Conclusion: The current findings indicate that the IL-17F H161R variant influences the risk of asthma and is a natural

IL-17F antagonist, suggesting a potential role for IL-17F in the etiology of asthma. (J Allergy Clin Immunol 2006;117:795-801.)

Key words: Asthma, candidate gene, case-control association analysis, IL-17F, single nucleotide polymorphism

Recent advances in human genome sequencing have facilitated discovery of novel genes. IL-17A was first cloned more than 10 years ago. Between 2000 and 2002, researchers discovered 5 additional members of the IL-17 gene family, primarily from analysis of genome sequences, and named them in the order of discovery (ie, IL-17B-F).¹ IL-17 family members belong to a distinct category of cytokine, and play a role in coordinating local tissue inflammation by inducing release of proinflammatory and neutrophil-mobilizing cytokines.¹ IL-17F, a recently discovered member of the IL-17 family,²⁻⁴ is produced by activated mast cells, CD4⁺ T cells, and basophils,² all of which are critically involved in airway inflammation of asthma. IL-17F induces several cell types (including bronchial epithelial cells) to express cytokines, chemokines, growth factors, and adhesion molecules, including IL-8, GM-CSF, growth-related oncogene α (GRO α), epithelial-cell derived neutrophil activating protein (ENA)-78, TGF- β , monocyte chemoattractant protein 1, and intercellular adhesion molecule 1.⁵⁻⁸ These molecules play a crucial role in leukocyte recruitment and activation, and remodeling of asthmatic airways. IL-17F induces activation of a signaling pathway involving Raf-1, mitogen-activated protein kinase kinase (MEK) 1 and 2 (1/2), and extracellular signal-regulated kinase (ERK) 1 and 2 (1/2); this is a central upstream signaling pathway for expression of IL-17F-induced cytokines and chemokines.^{2-4,9,10} Upregulated *IL17F* gene expression has been observed at sites of allergen challenge in the airways of patients with asthma.² In a murine model of asthma, IL-17F amplifies antigen-induced allergic inflammation.¹¹ Collectively, these findings indicate that IL-17F is involved in the pathophysiology of asthma.

The available evidence regarding the functions of IL-17F suggests that the *IL17F* gene is an excellent candidate gene for asthma susceptibility. In the current study, to examine the clinical relevance of IL-17F in asthma, we performed genetic case-control analyses of association

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Abbreviations used

ENA: Epithelial-cell derived neutrophil activating protein
 ERK: Extracellular signal-regulated kinase
 GRO α : Growth-related oncogene α
 MEK: Mitogen-activated protein kinase kinase
 muIL-17F: Mutated-type IL-17F
 OR: Odds ratio
 SNP: Single nucleotide polymorphism
 wtIL-17F: Wild-type IL-17F

between asthma and several *IL17F* gene polymorphisms, using 867 unrelated Japanese subjects. The current findings show that an *IL17F* gene polymorphism was inversely associated with the development of asthma. *In vitro* functional analysis revealed that the His-to-Arg substitution at amino acid 161 (H161R) in the 3rd exon of the *IL17F* gene causes loss of the ability of IL-17F to induce expression of certain cytokines and chemokines. The H161R variant of IL-17F antagonizes the function of wild-type IL-17F (wtIL-17F), and that antagonistic effect may underlie the association between the *IL17F* gene and asthma.

METHODS**Study population**

A total of 867 subjects were recruited. Subjects with bronchial asthma (subjects with asthma, $n = 432$) were recruited from the pulmonary clinic at the First Department of Medicine, Hokkaido University Hospital. All subjects with asthma were diagnosed with asthma by a doctor; had a history of coughing, wheezing, or dyspnea; and exhibited reversible airflow limitation (12% variability in either FEV₁ or peak expiratory flow rate, spontaneously or after inhalation of a short-acting β_2 agonist) and/or increased airway responsiveness to methacholine. We carefully excluded possible chronic obstructive pulmonary disease cases, using results of spirometric analysis (to check the reversibility of airflow obstruction) and x-ray/computed tomography examinations. Healthy controls ($n = 435$) were recruited from among people who requested annual physical examinations at Hokkaido University Hospital. All healthy controls had no history of bronchial asthma or other chronic lung diseases. We measured all subjects' total serum IgE levels (IU/mL) and assessed their specific IgE responses to 10 common inhaled allergens, including *Dermatophagoides farinae* (Der f), grass pollens, animal danders, and molds. An increase in specific IgE antibody levels (IgE CAP radioallergosorbent test > 0.35 UA/mL, or multiple antigen simultaneous test > 1.0 lumicount) was considered to be a positive response. We defined atopy as a positive response to at least 1 of the 10 common inhaled allergens, as described elsewhere.¹² All subjects were unrelated Japanese and gave written informed consent for enrollment in the study and all associated procedures. The Ethics Committee of the School of Medicine, Hokkaido University, approved the study.

Genotyping

Ten single nucleotide polymorphisms (SNPs) in the *IL17F* gene (−1507C/T, −394C/T, 1165A/G, 5045T/G, 5667A/G, 6328C/T, 7383T/C, 7469C/T, 7488T/C, and 8062C/G) were selected from

public databases using the bioinformatics tool SNPper¹³ (<http://snpper.chip.org>); nucleotide numbering starts from the first nucleotide of the transcription start site. The 10 SNPs included 3 polymorphisms in the coding region that cause amino acid substitutions: 7383T/C, 7469C/T, and 7488T/C. Initially, 50 control subjects (ie, 100 haploid genomes) were genotyped by direct sequencing for each polymorphism, and polymorphisms with minor allele frequencies > 0.1 were subjected to further analyses. The sequences of the primers used for direct sequencing are shown in this article's Table E1 in the Online Repository at www.jacionline.org.

Alleles were identified using an assay combining kinetic (real-time quantitative) PCR with allele-specific amplification, as described elsewhere.¹² Primers were designed using Primer Express software (PE Applied Biosystems, Foster City, Calif) to amplify specifically each SNP allele in separate PCRs. The sequences of the primers used for allele-specific PCR are shown in this article's Table E2 in the Online Repository at www.jacionline.org. Real-time PCR was performed using Syber Green I Master Mix (Applied Biosystems) and an ABI PRISM™ 7700 Sequence Detection System (Applied Biosystems).

Production of human recombinant wtIL-17F and mutated type IL-17F

Generation of human recombinant wtIL-17F is described in detail elsewhere.² The coding sequence of IL-17F was amplified by PCR and subcloned into the *Bam*HI and *Xba*I sites of the vector pcDNA 3.1 (Invitrogen, Carlsbad, Calif) to generate a C-terminal fusion gene with the histidine (His) and c-Myc tags. This pcDNA 3.1 was transfected into COS-7 cells using the Effectene reagent (Qiagen, Chatsworth, Calif) according to the manufacturer's instructions. Two days after transfection, the supernatants were concentrated over Centricon-10 columns (Amicon, Beverly, Mass) and subjected to affinity purification using Ni-nitrilotriacetic acid agarose beads (Qiagen) for His-tagged proteins. An expression construct containing the mutated type IL-17F (muIL-17F) sequence (H161R) was produced by T-to-C substitution using the QuikChange Site-Directed Mutagenesis kit (Stratagene, La Jolla, Calif) and using the wtIL-17F construct as a template. The sequence of each construct was confirmed using an ABI PRISM™ 7700 Sequence Detection System (Takara Syuzou, Shiga, Japan). Human recombinant muIL-17F was generated in a similar way. The concentrations of wtIL-17F and muIL-17F protein were quantified using the Bradford assay (BIO-RAD, Hercules, Calif). The wtIL-17F and muIL-17F proteins were stored at -80°C until used. Endotoxin levels were measured using Kinetic-QCL Chromogenic LAL (Bio Whittaker, Walkersville, Md); endotoxin levels were undetectable.

Detection of recombinant wtIL-17F and muIL-17F

To assay protein expression, wtIL-17F and muIL-17F (200 ng/lane) were suspended with an equal volume of 2 $\times$ loading buffer (0.1 mol/L Tris-HCl, pH 6.8, 4% SDS, 0.005% bromophenol blue, and 20% glycerol) containing 2-mercaptoethanol (0.7 mol/L), and were subjected to 4% to 20% Tris-glycine gel electrophoresis (NOVEX, San Diego, Calif). Gels were then transferred to polyvinylidene difluoride membranes (BIO-RAD) using a Trans Blot apparatus (NOVEX). The membranes were immersed overnight in TRIS-buffered saline/Tween 20 containing 5% nonfat dry skim milk (Carnation, Los Angeles, Calif). Immunoreactive proteins were detected using antibodies against IL-17F and His-tag. The antibodies used were rabbit anti-IL-17F Ab (R&D Systems, Minneapolis, Minn) and anti-His-tag Ab (Santa Cruz Biotechnology, Santa Cruz, Calif). The antibodies were suspended in TRIS-buffered saline/Tween 20 containing 5% skim milk for 1 hour. The membranes were then

TABLE I. Basic characteristics of the study subjects

	Patients with asthma	Controls	P value
Number of subjects	432	435	—
Sex (female/male)	235/197	154/281	<.001
Age (y; median, range)	47, 16-79	36, 18-72	<.001
Current smoker (%)	22.8%	26.2%	<.001
Atopy (%) [*]	74.3%	54.3%	<.001
Serum total IgE levels (log IU/mL, SD)	2.37 (0.67)	1.85 (0.63)	<.001
Age at onset of asthma (y; median, range)	33, 0-76	—	—

^{*}Atopy is defined as at least 1 positive RAST or MAST test; 10 common inhaled antigens were tested.

washed and incubated with peroxidase-linked donkey anti-rabbit-Ig Ab (Amersham Biosciences, Kashiwa, Japan) for 1 hour. The membranes were then washed again, and membrane-bound anti-rabbit-Ig Ab was visualized with enhanced chemiluminescence, using Western blotting detection reagents (Amersham Biosciences) and Hyper ECL luminescence detection film (Amersham Biosciences).

Cell culture and cytokine/chemokine analysis

BEAS-2B is a human airway epithelial cell line transformed with an adenovirus 12-simian virus 40 hybrid virus (donated by Dr Curtis Harris). BEAS-2B cells were cultured in Dulbecco modified Eagle medium (Biofluids, Inc, Rockville, Md) containing 10% FBS, 100 U/mL penicillin, and 100 ng/mL streptomycin (GIBCO BRL, Gaithersburg, Md), at 37°C in humidified air containing 5% CO₂. Levels of IL-8, GRO α , ENA-78, and GM-CSF in the supernatants of BEAS-2B cells incubated with wtIL-17F or muIL-17F were measured using a commercially available ELISA kit (Biosource, Camarillo, Calif) according to the manufacturer's instructions. Cell supernatants were harvested from cultures incubated with 10 or 100 ng/mL of wtIL-17F or muIL-17F for 24 or 48 hours. To assess whether muIL-17F interacts with the IL-17F receptor, competitive inhibition experiments were performed using the level of IL-8 expression as a functional readout. BEAS-2B cells were incubated with medium alone, medium containing wtIL-17F alone (100 ng/mL), or medium containing a combination of wtIL-17F (100 ng/mL) and varied doses of muIL-17F (1-1000 ng/mL) for 24 or 48 hours, and the level of IL-8 in the supernatant was measured.

Activation of Raf-1, MEK1/2, and ERK1/2

To investigate whether muIL-17F can activate Raf-1, MEK1/2, and ERK1/2, BEAS-2B cells were incubated with 100 ng/mL of wtIL-17F or muIL-17F for 20 minutes. After incubation, the cells were lysed in cold lysis buffer (20 mmol/L TRIS, pH 7.4, 4 mmol/L EDTA, 2 mmol/L ethyleneglycol-bis-(β -aminoethylether)-N,N,N',N'-tetraacetic acid, 1 mmol/L phenylmethylsulfonyl fluoride, 100 μ g/mL aprotinin, 200 μ g/mL leupeptin, 50 mmol/L NaF, 5 mmol/L Na₄P₂O₇, 1 mmol/L Na₃VO₄, and 1% Nonidet P-40; all purchased from Sigma [Tokyo, Japan]), and were then subjected to Western blotting analysis as described elsewhere.⁹ The antibodies used for Western blotting were rabbit anti-ERK1/2 Ab, anti-phospho-ERK1/2 Ab, anti-MEK1/2 Ab, and anti-phospho-MEK1/2 Ab, which were purchased from Cell Signaling Technology (Beverly, Mass), and anti-Raf-1 Ab and anti-phospho-Raf-1 Ab, which were purchased from Santa Cruz Biotechnology.

Data analysis

For genetic association analyses, each polymorphism was tested for deviations from Hardy-Weinberg equilibrium using the HWE

program (version 1.20; <http://linkage.rockefeller.edu/>). Pairwise linkage disequilibrium was estimated as r^2 . To evaluate linkage disequilibrium among 5 SNPs, we also used the Haplo.em function of the Haplo.Stats program (<http://linkage.rockefeller.edu/>),¹⁴ which calculates the likelihood ratio statistic for linkage disequilibrium by estimating haplotype frequencies.

Overall genotype frequencies were compared initially with the use of a $3 \times 2 \chi^2$ test with 2 *df*. If there was a significant overall difference between subjects with asthma and controls ($P < .01$), individual genotypes were compared using a $2 \times 2 \chi^2$ test with 1 *df*. Note that these statistical tests were not independent because of the linkage disequilibrium between the 5 polymorphisms, and applying significance levels of 0.01, the association between each polymorphism and asthma was significant at $P = .05$. The odds ratios (ORs) and 95% CIs were calculated using the Sheehe correction,¹⁵ because none of the patients with asthma were homozygous for the H161R variant. To allow for possible confounding effects of atopy, $2 \times 2 \chi^2$ analysis was also performed using the Mantel-Haenszel test and stratification according to atopic status. The Haplo.score function of the Haplo.Stats program¹⁴ was used to test for statistical association between the *IL17F* haplotypes and asthma, and simulation *P* values were computed for each haplotype.

For the functional analysis of muIL-17F, the statistical significance of differences was assessed by using ANOVA. The values are expressed as means $\pm$ SDs from independent experiments. Differences with *P* values $< .05$ were considered significant. When ANOVA indicated a significant difference, the Scheffé *F* test was used to determine the significance of the difference between subjects with asthma and controls.

RESULTS

The demographic characteristics of the 435 healthy controls and 432 subjects with asthma are shown in Table I. The mean age was highest for nonatopic patients with asthma, most of whom were women. Subjects with asthma had higher levels of total serum IgE than the controls (*t* test, $P < .01$). Fewer subjects with asthma than controls were current smokers (22% vs 25%; χ^2 test, $P < .01$). More than 50% of the control subjects were atopic, which is consistent with recent findings that the prevalence of atopy (as indicated by specific IgE against common inhaled allergens) among Japanese is increasing.^{16,17}

In initial genotyping of 10 *IL17F* polymorphisms by direct sequencing, 5 SNPs (−394C/T, 5667A/G, 7383C/T [E126G], 7469C/T [V155I], and 8062C/G) were not polymorphic (at least in the first 100 chromosomes

TABLE II. Genotype frequencies of *IL17F* polymorphisms in patients with asthma and controls

Markers	Genotype	Asthma	Control	Maf*	P value†
-1507 C/T rs1889570	CC	250	253	0.239	.098
	CT	140	156		
	TT	42	26		
1165 A/G rs1266828	AA	276	271	0.211	.78
	AG	134	144		
	GG	22	20		
5045 C/T‡ rs7771511	CC	335	353	0.101	.041
	CT	96	76		
	TT	1	6		
6328 C/T rs766748	CC	243	262	0.226	.24
	CT	154	149		
	TT	35	24		
7488 T/C‡ His → Arg rs763780	TT	332	347	0.111	.0028
	TC	100	79		
	CC	0	9		

*Allele frequency of the minor allele in controls

†P value from a genotype-based χ^2 test of case-control data‡The Fisher exact method was used for 5045C/T and 7488T/C polymorphisms in 2×2 tables. The probabilities of association between these polymorphisms and asthma were 0.124 for 5045C/T (CC or CT vs TT) and 0.0038 for 7488T/C (TT or TC vs CC).**TABLE III.** Haplotypes of *IL17F*

Haplotypes*	Frequency†‡		
	Total	Asthma	Control
CACCT	0.395	0.381	0.410
CGCCT	0.195	0.191	0.197
TACTT	0.187	0.200	0.175
CATCC	0.095	0.105	0.085
TACCT	0.051	0.048	0.054
CACTT	0.040	0.048	0.034

*Haplotypes (-1507 C/T, 1165 A/G, 5045 C/T, 6328 C/T, 7488 T/C) with frequency > 0.01 are presented.

†Frequency of each haplotype in 867 subjects was estimated. Analysis using the Haplo.em program showed that these 5 polymorphisms were in strong linkage disequilibrium (likelihood ratio statistic = 924.2; $df = 14$; $P < .000001$).

‡None of haplotypes was associated with asthma.

sequenced). The other 5 SNPs (1507C/T, 1165A/G, 5045C/T, 6328C/T, and 7488T/C [H161R]) had minor allele frequencies > 10% among the 435 controls (Table II). The latter 5 SNPs were in strong linkage disequilibrium with each other, and their distribution was in Hardy-Weinberg equilibrium for controls. In contrast, among patients with asthma, we found significant deviations from the expected proportions of the genotypes of -1507C/T ($\chi^2 = 10.55$; $P = .0012$), 5045C/T ($\chi^2 = 4.76$; $P = .029$), and 7488T/C ($\chi^2 = 7.4$; $P = .0065$). Note that the proportions of -1507CT heterozygote, 5045TT homozygote, and 7488CC homozygote were significantly lower than the expected proportions under Hardy-Weinberg equilibrium (calculated from Table II); this may be a result of association with the disease allele. Six common haplotypes (frequency > 0.01) were constructed along with the *IL17F* gene, accounting for >96% of the distribution (Table III). We also used the program LDPlotter (<http://www.innateimmunity.net/IIPGA2/Bioinformatics/>) to plot the linkage disequilibrium pattern, using r^2 for 5 SNPs in the asthma and control groups (see this article's Fig E1 in the Online Repository at www.jacionline.org).

Genotype-based χ^2 association analyses indicated that the 7488T/C polymorphism was significantly associated with asthma ($\chi^2 = 11.8$, $df = 2$; $P = .0028$; Table II). It is noteworthy that none of the patients with asthma were homozygous for the H161R variant; the OR for asthma was 0.06 (95% CI, 0.01-0.43; $P = .0039$) for the H161R homozygote, compared with the homozygote for the wild-type allele (Table IV). In contrast, heterozygosity for the H161R variant was not associated with asthma, compared with the wild-type (His161) homozygote (OR, 1.32; 95% CI, 0.95-1.84; Table IV). After adjusting for atopy, the inverse association between the H161R homozygote and asthma remained significant (Mantel-Haenszel $\chi^2 = 7.05$ for the H161R homozygote; $P = .0079$).

Because demographic factors including sex, age, and smoking status differed significantly between patients with asthma and healthy controls, we also conducted 2×2 χ^2 analysis using the Mantel-Haenszel test to examine association between the *IL17F*7488 polymorphism (CC vs TC or TT) and asthma, allowing for possible confounding effects of these covariates. After adjusting for sex (male/female), age (<40 y/≥40 y) or smoking status (current smoker, exsmoker, or never smoked), the inverse association between the H161R homozygote and asthma remained significant (Mantel-Haenszel $\chi^2 = 6.0$ [$P = .014$], 5.7 [.017], and 6.6 [.011] when adjusted for sex, age, and smoking status, respectively). In contrast, none of the haplotypes was significantly associated with asthma (Table III).

His-tagged muIL-17F protein expressed in COS-7 cells reacted with anti-IL-17F Abs and anti-His-tag Abs (Fig 1, A). Unlike wtIL-17F, muIL-17F did not induce activation of Raf-1, MEK1/2, or ERK1/2 in bronchial epithelial cells

TABLE IV. Association between the 7488T/C polymorphism (H161R) and asthma

	OR (95% CI)	P value*	
		Unadjusted	Adjusted†
TT	1	Reference	Reference
TC	1.32 (0.95-1.84)	.11	.08
CC	0.06 (0.01-0.43)	.0039	.0079

*P values were calculated using the χ^2 test with 1 df.

†The adjusted value was obtained using the Mantel-Haenszel test after stratification according to atopic status.

(Fig 1, B). As expected, wtIL-17F induced production of significant levels of cytokine (GM-CSF) and chemokines (IL-8, GRO α , and ENA-78) 24 and 48 hours after incubation, whereas muIL-17F did not induce expression of those proteins (Fig 1, C). There was no statistical difference in levels of cytokines or chemokines between incubation with muIL-17F (both doses) and incubation with medium only (controls).

The loss-of-function of muIL-17F was not a result of its inability to bind to the IL-17F receptor, because muIL-17F blocked wtIL-17F-induced IL-8 production in a dose-dependent manner (Fig 2). At equal concentrations of muIL-17F and wtIL-17F (100 ng/mL), muIL-17F inhibited about 50% of the wtIL-17F-induced IL-8 production. This inhibition reached 100% when the concentration of muIL-17F was 5-fold higher than that of wtIL-17F, suggesting that muIL-17F is a naturally occurring antagonist of IL-17F.

DISCUSSION

The current findings demonstrate that homozygosity for the Arg161 variant of the IL-17F H161R polymorphism is inversely associated with development of asthma, and is associated with impaired IL-17F signaling *in vitro*. Allergen challenge induces gene expression of *IL17F* in human asthmatic airways.² IL-17F induces expression of several CXC chemokines and cytokines including proinflammatory cytokines such as IL-6 and TGF- β . Overexpression of IL-17F in a mouse model with increased numbers of neutrophils in the airways has an additive effect on antigen-induced allergic inflammatory responses, such as increased airway hyperresponsiveness, goblet cell hyperplasia, and mucin gene expression, which are involved in the pathophysiology of allergic inflammation and airway remodeling.¹¹ The current results of *in vitro* analyses suggest that the H161R variant of IL-17F has less ability to potentiate allergic inflammation and remodeling processes in the airways, and thus is associated with reduced risk of developing asthma. The finding that the H161R variant of IL-17F acts as a natural antagonist of IL-17F suggests that targeting IL-17F activity has potential as a basis of therapy.

Fig 2 shows that the Arg161 variant acted as an antagonist of wtIL-17F at a 1:1 ratio, but there was no protective

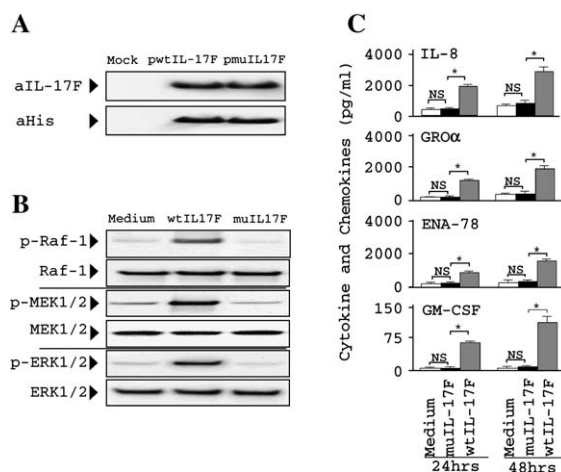


FIG 1. A, Detection of recombinant wtIL-17F and muIL-17F proteins by Western blotting. B, Phosphorylation of Raf-1, MEK1/2, and ERK1/2 induced by wtIL-17F or muIL-17F. Western blotting was performed as described in Methods. C, Expression of cytokine and chemokine induced by wtIL-17F or muIL-17F. The values are expressed as means $\pm$ SDs (n = 6). *P < .05 was considered to indicate significance, versus medium alone.

effect against asthma in 7488TC heterozygotes. Thus, the finding that IL-17F stimulates IL-8 production *in vitro* may not fully account for the association between the H161R genotype and asthma. Asthma is a complex genetic disease that involves multiple genes, with both genetic heterogeneity and a threshold effect for disease expression. The genetic model typically presented is one in which “affected” individuals are those that lie above some biological threshold of risk; asthma begins when an individual’s liability exceeds the asthma threshold; environmental and other causative factors may increase or decrease an individual’s asthma liability. We assume it is unlikely that overall disease liability of H161R homozygotes (but not heterozygotes) easily exceeds the clinical asthma threshold. Another possible explanation is that IL-17F-stimulated IL-8 production *in vitro* does not reflect local tissue IL-8 levels in the airways. In the current study, levels of IL-8 were measured after a short period ($\sim$ 48 h) of stimulation by IL-17F *in vitro*; repeated, longitudinal stimulation may be necessary to induce a marked, sustained effect on the pathophysiology of asthma *in vivo*.

In a recent study by Ramsey et al,¹⁸ relationships between asthma and *IL17F* polymorphisms (including the H161R variant) were examined using 1027 white female subjects. The study found no associations between asthma and *IL17F* polymorphisms. There have been reports of such discrepancies in associations of asthma for many candidate gene loci. The discrepancy between the European Americans studied by Ramsey et al¹⁸ and the current Japanese subjects may be a result of genetic heterogeneity and differences in environmental factors, including socioeconomic factors. Furthermore, the frequency of the H161R variant was lower for the European American subjects (4.5%) than for the current Japanese subjects (11.4%), suggesting that the study by Ramsey et al¹⁸ did

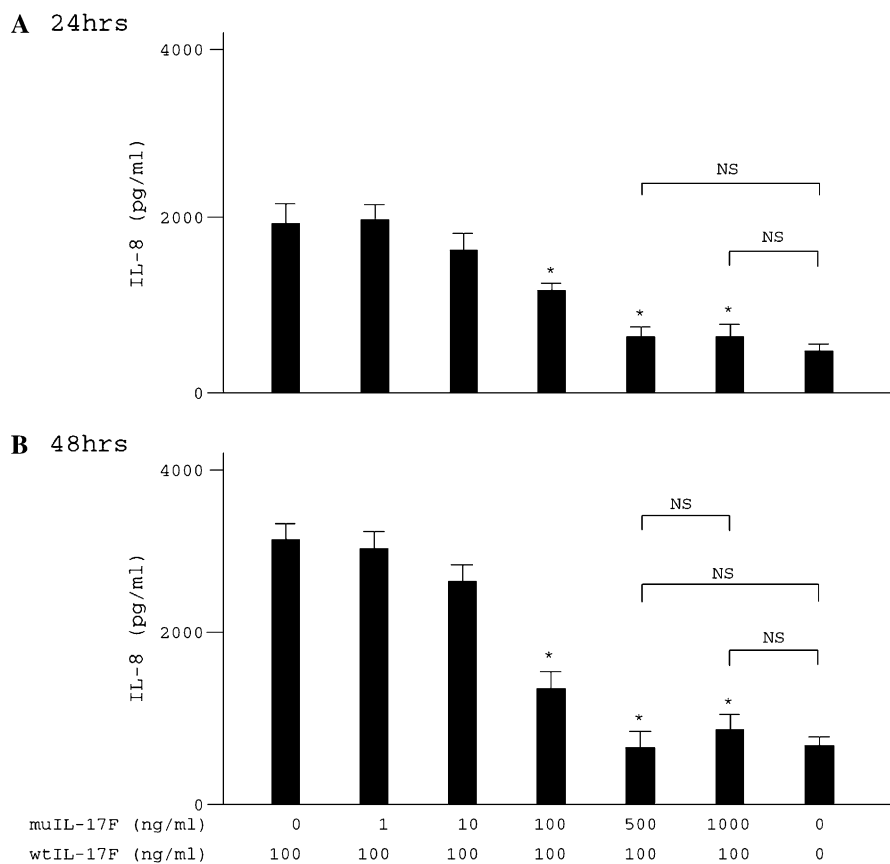


FIG 2. Effect of muIL-17F on wtIL-17F-induced IL-8 expression on BEAS-2B cells. BEAS-2B cells were incubated with medium alone, medium containing wtIL-17F alone, or medium containing a combination of wtIL-17F and varied different doses of muIL-17F for (A) 24 and (B) 48 hours. The values are expressed as means $\pm$ SDs (n = 4). * P < .05 was considered to indicate significance, versus wtIL-17F (100 ng/mL) alone.

not have enough power to detect genetic effects of the H161R variant.

An important current finding is that the frequency of the H161R homozygote was low (1.0%) compared with the frequency of the H161R heterozygote (20.6%). This may explain why there was no difference in the frequency of the haplotype carrying the 7488C allele between patients with asthma and controls, because the H161R homozygote but not the H161R heterozygote had a significantly decreased risk of asthma. The low frequency of the H161R homozygote also suggests that the H161R variant does not contribute substantially to the disease at the population level. There are competing theories of complex disease inheritance. One popular hypothesis proposes that the genetic factors underlying common diseases are alleles that are quite common in the general population.¹⁹ In contrast, the rare variant hypothesis proposes that rare variants are collectively responsible for a substantial proportion of multifactorial inherited susceptibility to a variety of chronic diseases.²⁰ The current findings suggest that the H161R homozygote is one of the rare variants that collectively influence asthma susceptibility, and are thus consistent with the rare variant hypothesis. Nevertheless, given the current findings of analyses of individual

SNPs and *in vitro* functional analyses, the H161R variant appears to be the most likely underlying cause of the association between the *IL17F* region and asthma, although we cannot exclude the possibility of linkage disequilibrium between the H161R variant and other neighboring genes without additional fine-mapping analysis of SNPs throughout the region.

The IL-17F receptor has not been identified, but indirect evidence suggests that it is a distinct cognate receptor.² In the current competitive inhibition experiments, the level of wtIL-17F-induced IL-8 expression was significantly decreased by several concentrations of muIL-17F. The crystal structure of IL-17F has recently been determined, and IL-17F is believed to form disulfide-bonded homodimers consisting of 2 pairs of antiparallel β -strands.⁴ The H-to-R substitution at amino acid 161 is closer to the C-terminal end of the IL-17F sequence, which is not part of the β -strand structure. The structural basis for the antagonistic activity of muIL-17F is unclear, but it is unlikely that the H-to-R substitution at amino acid 161 would affect the core conformation of the protein. However, that substitution may influence the affinity of binding to its receptor. Mutant IL-17F did not activate the Raf-1-MEK1/2-ERK1/2 pathways, despite antagonizing wtIL-17F

function; this suggests that muIL-17F binds to the IL-17F receptor but does not activate its signaling pathway. Further study is needed to clarify the mechanisms by which muIL-17F exerts its antagonistic effect.

In conclusion, the results of the current genetic analysis and *in vitro* functional analysis provide the first evidence that the *IL17F* coding variant H161R, which is associated with impaired IL-17F signaling, protects against asthma in Japanese. These findings suggest that this variant is functional *in vivo*, and that it influences asthma susceptibility. Further studies are needed to identify the exact mechanism underlying the involvement of the *IL17F* gene in the pathogenesis of asthma.

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