

Functional haplotypes of *IL-12B* are associated with childhood atopic asthma

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Background: IL-12 is a heterodimeric proinflammatory cytokine that forms a link between innate and adaptive immunity. Although associations between polymorphisms of *IL-12B* on chromosome 5q31-33 and asthma have been reported, the genetic influences of the polymorphisms and haplotype of *IL-12B* are unclear.

Objective: To examine whether polymorphisms in *IL-12B* are associated with childhood atopic asthma in a Japanese population.

Methods: We identified a total of 13 polymorphisms and characterized the linkage disequilibrium mapping of the gene. Three variants in the promoter and 3' untranslated region were genotyped, and we conducted case-control and case-only association studies between those variants and asthma-related phenotypes (childhood atopic asthma, $n = 297$; normal controls, $n = 555$). Haplotype association analysis and functional analysis of these variants were also performed.

Results: 3' Untranslated region 10841C>A was significantly associated with the risk of childhood atopic asthma ($P = .00062$). The -6415 promoter variant, in linkage disequilibrium with the 10841C>A ($D' = 0.78$ and $r^2 = 0.61$), was also marginally associated with childhood atopic asthma ($P = .051$). We analyzed the 2-locus haplotype by using these

2 polymorphisms and found a positive association with haplotype CTCTAA-C (-6415 CTCTAA and 10841C; $P = .00078$). Furthermore, 10841C>A affects the stability of transcripts, and promoter variant -6415GC enhances the transcriptional level of *IL-12B*.

Conclusion: Our results imply that functional haplotype CTCTAA-C, which affects the instability of transcripts and the lower transcriptional level of *IL-12B*, has a protective effect in childhood atopic asthma. On the basis of these findings, the *IL-12B* gene might be involved in the development of atopic asthma through functional genetic polymorphisms. (J Allergy Clin Immunol 2005;116:789-95.)

Key words: Asthma, IL-12B, polymorphism, association, linkage disequilibrium, haplotype

Asthma is defined as a chronic inflammatory lung disease characterized by airway hyperreactivity and mucus hypersecretion that results in intermittent airway obstruction.¹ A growing body of evidence suggests that 2 subsets of T_H cells, T_H1 and T_H2 , may play important roles in allergic disorders.^{2,3} Although IL-12 is an immunomodulatory cytokine that is the primary inducer of the development of T_H1 cells with downregulation of T_H2 cytokines,^{4,5} recent studies have shown that p80 (a p40 homodimer) and the p40 monomer have important roles in T_H2 -type immune responses.⁶⁻⁹ Because of alternative heterodimeric partnering and monomer secretion, IL-12 p40 encoded by *IL-12B* is a component of 4 secreted proteins: IL-12 (a p40 and p35 heterodimer),⁵ IL-23 (a p40 and p19 heterodimer),¹⁰ p80 (a p40 homodimer), and p40 (a p40 monomer).⁶⁻⁹ Each protein has distinct biologic properties. There is strong evidence that IL-12 p40 is crucial for the etiology of asthma and for the induction of T_H2 -type allergic inflammation in an animal model.¹¹⁻¹³ Recent studies have indicated that IL-12 p40 may function as an antagonist of IL-12 action.⁶⁻⁹ Furthermore, increased IL-12 p40 homodimer (p80) levels are associated with enhanced airway macrophage accumulation and overexpression of IL-12 p40 selectivity in airway epithelial cells in subjects with asthma.¹³

IL-12B locates in 5q31.1-33.1, which is linked to asthma susceptibility and asthma-related phenotypes,¹⁴⁻¹⁸ and is also one of the most consistently replicated regions

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Abbreviations used

LD: Linkage disequilibrium
OR: Odds ratio
SNP: Single nucleotide polymorphism
tIgE: Total IgE
UTR: Untranslated region

in diverse populations. In the Japanese population, convincing evidence of linkage to atopic asthma susceptibility on chromosome 5q has been observed by sibling pair analysis.¹⁴ Several polymorphisms have been identified in the *IL-12B* gene, and genetic studies have been conducted for these polymorphisms.¹⁹⁻²¹ It is important to determine whether the finding is replicable in other ethnic or independent populations and examine the roles of the associated variants in the development of the asthma phenotype.

To test whether variants of *IL-12B* were related to asthma, we first performed linkage disequilibrium mapping of the gene and conducted an association study and haplotype analyses with regard to the linkage disequilibrium (LD) pattern. In addition, we performed functional analyses of the associated polymorphisms.

METHODS**Study subjects**

All subjects with asthma were diagnosed according to the criteria of the National Institutes of Health²² and demonstrated at least 12% improvement in their FEV₁ measurement after β_2 -agonist inhalation. The diagnosis of atopic asthma was based on 1 or more positive skin scratch test responses to seven common aeroallergens in the presence of a positive histamine control and a negative vehicle control. The seven aeroallergens were house dust, *Felis domesticus* dander (Fel d), *Canis familiaris* dander, *Dactylis glomerata*, *Ambrosia*, *Cryptomeria japonica* and *Alternaria alternata*. Peripheral blood was obtained from each of 297 pediatric atopic outpatients with asthma at the Osaka Prefectural Habikino Hospital and National Sagami Hospital (mean age, 9.6 years; range, 3-15 years; male:female ratio, 1.63:1.0; mean serum IgE level, 490 IU/mL; *Dermatophagoides pteronyssinus* or *Dermatophagoides farinae* RAST-positive, 80.0%). Specific IgE was considered positive when values exceeded 0.35 UA (arbitrary units)/mL (RAST score ≥ 1) according to an enzyme immunoassay. Serum IgE levels were log₁₀-transformed before analyses. The mean of log₁₀(total IgE [tIgE] [IU/mL]) of patients with childhood atopic asthma was 2.69 (= log₁₀[490 IU/mL]). In this study, high IgE levels were defined as those values in the 75th percentile or higher for total IgE. The 75th percentile value of log₁₀ (tIgE) in patients with childhood atopic asthma was 3.04 (= log₁₀[1106 IU/mL]).²³ The severity of asthma was defined according to the degree of therapy required to control symptoms at the time of entry into the study. The grades were as follows: grade 1, β -agonists only; grade 2, sodium cromoglycate and/or theophylline; grade 3, inhaled beclomethasone 400 μ g/d or less; grade 4, inhaled beclomethasone of more than 400 μ g/d.²³ A total of 555 healthy individuals who had neither respiratory symptoms nor a history of asthma-related diseases (mean age, 45 years; range, 18-75 years; male:female ratio, 2.44:1.0) were recruited by physicians' interviews about whether they had been diagnosed with asthma and/or atopy. Because there was a

large age difference between the cases and controls, we performed linear regression analysis between age and allele frequencies of genotyped single nucleotide polymorphisms (SNPs). R^2 values of all SNPs were less than 0.001, so there was no evidence of association between age and allele frequencies. All individuals were Japanese and gave written informed consent to participate in the study (or, for individuals younger than 16 years, their parents gave consent) according to the rules of the process committee at the SNP Research Center, Institute of Physical and Chemical Research (RIKEN).

Screening for polymorphisms and genotyping

To identify polymorphisms in the human *IL-12B* gene, we sequenced all 8 exons, including a minimum of 100 bases of the flanking intronic sequence, 2.98 kb of the 5' flanking region, and a 0.2-kb continuous 3' flanking region of the 8th exon from 24 subjects with asthma and 12 control subjects. Fourteen primer sets were designed on the basis of the *IL-12B* genomic sequence from the GenBank database (accession number AC011418.5; see Table E1 in the Online Repository in the online version of this article at www.jacionline.org). The sequences were analyzed and polymorphisms identified by using the SEQUENCHER program (Gene Codes Corp, Ann Arbor, Mich). The promoter polymorphisms, -6415 CTCTAA>GC and -5741G>C, were genotyped by use of the TaqMan system (Applied Biosystems, Foster City, Calif). For the 10841C>A 3' untranslated region (UTR) polymorphism, genotyping was performed by PCR-RFLP analysis by using *Taq* I as described.²⁴

Luciferase assay

The human embryonic kidney 293 cell line was obtained from the RIKEN cell bank. Three concatenated copies of the 20-bp or 16-bp DNA fragments were cloned into pGL3-basic vector (Promega, Madison, Wis) in the 5'-3' orientation. The DNA fragments were as follows: for -6415CTCTAA, 5'-AGAGAGGCTCTAATGTGGCC-3', and -6415GC, 5'-AGAGAGGGCTGTGGCC-3'. We then transfected subconfluent 293 cells (1×10^6) cultured in 12-well plates with 0.5 μ g of each construct and 0.01 μ g pRL-TK Renilla luciferase vector (Promega), an internal control for transfection efficiency, using 1.5 μ L FuGENE 6 transfection reagent (Roche Diagnostics, Basel, Switzerland). After 24 hours, we lysed cells and measured luciferase activities in a luminometer by using the Dual-Luciferase Reporter Assay System. The relative luciferase activity of the *IL-12B* reporter constructs was represented as the ratio of the firefly luciferase activity to that of Renilla. Each experiment was repeated 3 times, and each sample was studied in triplicate as described.²⁵

Stability of 2 types of *IL-12B* mRNA

We amplified genes encoding 2 *IL-12B* variants by PCR from cDNAs synthesized by using a first-strand cDNA synthesis kit (Invitrogen, Carlsbad, Calif) with PBMC total RNA. We cloned these amplified genes into the pENTR vector (Invitrogen) and constructed cDNA in pDEST14 (Invitrogen). After digestion with ClaI and *in vitro* expression using RiboMax Large Scale RNA Production System-T7 (Promega), we mixed and incubated 0.5 μ g of each synthesized RNA and diluted whole-cell extracts of U937 cells (1:1500) at room temperature as described.²⁶ We detected RNA by using Northern blot hybridization and measured signal intensities of full-length RNAs.

Statistical analysis

We calculated allele frequencies and tested agreement with Hardy-Weinberg equilibrium by using a χ^2 goodness-of-fit test at each locus. We then compared differences in allele frequencies and genotype distribution of each polymorphism between case and

TABLE I. Locations and allele frequencies of polymorphisms in *IL-12B* screened with 36 Japanese subjects*

Polymorphism	Location	Sequence	Allele frequency (%)†	JSNP (IMS-JST)	NCBI
–6415 CTCTAA/GC	5'g	5'-AGAGG(CTCTAA/GC)TGTGG-3'	53	—	—
–6110 C/T	5'g	5'-CACTG(C/T)GGGAA-3'	50	—	rs2546890
–5741 G/C	5'g	5'-TGGTG(G/C)AGGTG-3'	18	—	—
–5027 G/A	5'g	5'-GGGAG(G/A)AAGTG-3'	2	—	—
–3624 C/T	Intron1	5'-TACCT(C/T)CCTCC-3'	2	—	—
–3312 G/A	Intron1	5'-TAAAG(G/A)ATTGT-3'	2	—	—
1521 A/G	Intron2	5'-TTAGC(A/G)AGCTC-3'	55	132913	rs3181217
1645 A/G	Intron2	5'-ACGAT(A/G)CAGTG-3'	55	132914	rs3181218
3778 G/A	Intron3	5'-ATAAG(G/A)GTAA-3'	55	040193	rs2288831
6557 G/A	Intron5	5'-TGTGG(G/A)GTAGG-3'	2	—	—
9945 C/T	Intron6	5'-CTTCA(C/T)AGCTT-3'	2	—	rs11574790
10559 WT/delG	Intron7	5'-AGGAA(WT/delG)CCTGG-3'	55	—	rs3213111
10841 C/A	Exon8	5'-TAGTT(C/A)GATGC-3'	55	—	rs3212227

JSNP, Number from the Japanese SNP database (<http://snp.ims.u-tokyo.ac.jp/>); IMS-JST, Institute of Medical Science-Japan Science and Technology Agency; NCBI, number from the dbSNP of National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/SNP/>).

*Positions are numbered according to their position relative to the published *IL-12B* gene-containing clone (GenBank AC011418.5). Position 1 is the A of the initiation codon.

†Frequency of right indicated allele.

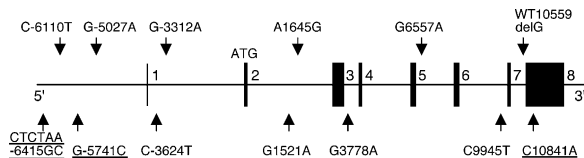


FIG 1. Graphical overview of polymorphisms identified in relation to the exon/intron structure of the human *IL-12B* gene. Eight exons are shown by black boxes with their numbers, and positions for polymorphisms are relative to the translation start site (+1). Underlined polymorphisms were genotyped in the whole samples.

control subjects by using a 2×2 contingency χ^2 test with 1 *df* and calculated odds ratios (ORs) with 95% CIs. Pairwise LD was calculated as $|D'|$ and r^2 by using the SNP Alyze statistical package (Dynacom, Chiba, Japan) as described.²⁷ To infer the frequencies of haplotypes in control and patient groups and diplotypes in individuals with uncertain phases of genotypes, software programs PHASE version 2.0.2 (<http://www.stat.washington.edu/stephens/software.html>)²⁸ (University of Washington, Seattle, Wash) and HAPLOTYPER (<http://www.people.fas.harvard.edu/~junliu/Haplo/docMain.htm>)²⁹ (Harvard University, Cambridge, Mass) were used. Haplotype frequencies in cases and controls were evaluated both by the whole distribution with the Fisher exact test and by χ^2 tests of one haplotype against others (haplotype-wise test). We applied Bonferroni corrections, the multiplication of *P* values by the number of variants. Corrected *P* values of less than .05 were judged to be significant. Comparisons in reporter assays as well as mRNA degradation assays were performed with the Student *t* test. A *P* value of less than .05 was considered statistically significant.

RESULTS

We identified 13 biallelic polymorphisms in *IL-12B*: 4 in the 5' flanking region, 1 in the 3' UTR, and 8 in the intron (Table I and Fig 1). Seven polymorphisms were contained in the public databases available at Web sites. 10841C>A in exon 8 was reported previously as 1188C>A.²⁴ Rare SNPs with minor allele frequencies of 2% were not included in the analysis. Pairwise LD among

TABLE II. Pairwise linkage disequilibrium for all possible 2-way comparisons among 8 polymorphisms in *IL-12B* with 36 Japanese subjects

	SNP1 –6415 CTCTAA/GC*	SNP2 –6110 C/T	SNP3 –5741 G/C*	SNP4 1521 A/G	SNP5 1645 A/G	SNP6 3778 G/A	SNP7 10559 WT/delG	SNP8 10841 C/A*
SNP1	D'	1.00	1.00	0.78	0.78	0.78	0.78	0.78
	r^2	0.87	0.20	0.61	0.61	0.61	0.61	0.61
SNP2		D'	1.00	0.74	0.74	0.74	0.74	0.74
		r^2	0.22	0.45	0.45	0.45	0.45	0.45
SNP3			D'	0.35	0.35	0.35	0.35	0.35
			r^2	0.02	0.02	0.02	0.02	0.02
SNP4				D'	1.00	1.00	1.00	1.00
				r^2	1.00	1.00	1.00	1.00
SNP5					D'	1.00	1.00	1.00
					r^2	1.00	1.00	1.00
SNP6						D'	1.00	1.00
						r^2	1.00	1.00
SNP7							D'	1.00
							r^2	1.00

*Polymorphisms were genotyped in this case-control study.

8 SNPs with a frequency >0.15 was measured by different parameters, $|D'|$ and r^2 (Table II). D' is inversely biased with sample size, and the degree of bias will be greater for SNPs with lower allele frequencies. In contrast, r^2 is highly dependent on allele frequency.³⁰ There is no single best measure of LD under all possible situations; we used complementary measures, D' and r^2 .³⁰ The promoter variant, SNP1, was in strong LD with another promoter variant, SNP2 –6110C>T ($D' = 1.00$ and $r^2 = 0.87$). Although SNP3 –5741G>C was in LD with SNP1 when it was evaluated by $D' (= 1.00)$, the R^2 value of LD between SNP1 and SNP3 was not so high ($r^2 = 0.20$). The 3' UTR variant 10841C>A was in complete LD ($D' = 1.00$ and $r^2 = 1.00$) with 1521A>G, 1645A>G, 3778G>A, and 10559WT>delG (Table II). We finally selected 3 polymorphisms with minor allele frequencies of more than

TABLE III. Association between polymorphisms of *IL-12B* and childhood atopic asthma

Locus	Allele1/2	Genotype	Childhood atopic asthma (%)	Control (%)	P value	OR (95% CI)
SNP 1 -6415	CTCTAA/GC	11	72 (25)	162 (30)	.051*†	1.28 (1.05-1.57)
		12	132 (46)	273 (50)		
		22	81 (28)	112 (20)		
SNP 3 -5741	G/C	11	176 (64)	340 (62)	.55	1.08 (0.84-1.40)
		12	88 (32)	194 (35)		
		22	10 (4)	19 (3)		
SNP 8 10841	C/A	11	68 (23)	175 (32)	.00062*†	1.46 (1.20-1.79)
		12	144 (49)	273 (50)		
		22	84 (28)	101 (18)		

*P value corrected with Bonferroni correction (raw P values were multiplied by 3).

†Allele1 vs allele2.

TABLE IV. Frequencies of haplotypes and ORs in the control group and childhood atopic asthma group

Haplotypes			Childhood asthma		Control		OR (95% CI)	χ^2 (df = 1)	P value*
SNP1 CTCTAA/GC	SNP8 C/A	Number of allele	Ratio	Number of allele	Ratio				
1	CTCTAA	C	243	0.409	559	0.504	0.68 (0.56-0.84)	13.9	.00078
2	GC	A	275	0.463	433	0.390	1.35 (1.10-1.65)	8.46	.015
3	GC	C	37	0.062	70	0.063	0.99 (0.65-1.49)	0.00	>1
4	CTCTAA	A	39	0.066	48	0.043	1.56 (1.01-2.40)	4.01	.18
Total allele			594		1110				

*P value corrected with Bonferroni correction (raw P values were multiplied by 4).

TABLE V. Frequencies of diplotypes and ORs in the control group and in the asthma group

Name	Number	Diplotype frequencies			OR (95% CI)	χ^2 (df = 1)	P value*
Haplotype 1		Homozygote	Heterozygote	Others			
Childhood asthma	297	0.178	0.461	0.360			
Controls	555	0.256	0.495	0.249	0.59 (0.43-0.80)†	11.8†	.0054†
Haplotype 2		Homozygote	Heterozygote	Others			
Childhood asthma	297	0.222	0.481	0.296			
Controls	555	0.141	0.499	0.360	1.75 (1.22-2.51)‡	9.20‡	.022‡

*P value corrected with Bonferroni correction (raw P values were multiplied by 9).

†Haplotype1/haplotype1 + haplotype1/others vs others/others.

‡Haplotype2/haplotype2 vs haplotype2/others + others/others.

15%, SNP1 promoter variant CTCTAA>GC, SNP3 -5741G>C, and SNP8 3' UTR variant 10841C>A.

All of these loci were in Hardy-Weinberg equilibrium in the control group. The allele frequency of each selected polymorphism was compared between the patients and the normal controls by the χ^2 test using codominant, dominant, and recessive models. After correcting for the number of variants investigated (Bonferroni correction), we found a significant association between the 3' UTR polymorphism at 10841 and childhood atopic asthma with the codominant model ($P = .00021$; corrected $P = .00062$). The promoter polymorphism, -6415 CTCTAA>GC, was marginally associated with childhood atopic asthma ($P = .017$; corrected $P = .051$). There was no significant association between SNP3 -5741G>C and childhood atopic asthma (Table III).

We further analyzed the haplotype structure by using 2 SNPs, SNP1 and SNP8, and associations of each of the 4 haplotypes with childhood atopic asthma as described (Table IV).³¹ PHASE tests the null hypothesis that the case and control haplotypes are a random sample from a single set of haplotype frequencies, versus the alternative that cases are more similar to other cases than to controls. We obtained a P value of .0011 with 10,000 permutations and found a positive association with haplotype CTCTAA-C ($P = .00039$; corrected $P = .00078$; haplotype 1 vs others; Table IV). As shown in Table V, we examined the association of the diplotypes of *IL-12B* with asthma. The results suggested that a homozygote or heterozygote of haplotype 1 showed a lower risk for childhood atopic asthma (OR, 0.59; 95% CI, 0.43-0.80) and a homozygote of haplotype 2 showed a higher risk for

TABLE VI. Association between polymorphisms of *IL-12B* and childhood atopic asthma with related phenotype*

SNP	Samples	Genotype (%)			P†	OR (95% CI)
		11	12	22		
SNP1	Control	547	162 (30)	273 (50)	112 (20)	
–6415	Severity ≤ grade 2	156	42 (27)	68 (44)	46 (29)	
CTCTAA /GC	Severity ≥ grade 3	112	28 (25)	52 (46)	32 (29)	
	IgE < 1106 IU/mL	146	32 (22)	62 (42)	52 (36)	
	IgE ≥ 1106 IU/mL	116	40 (34)	70 (60)	29 (25)	.020‡ 1.54 (1.11-2.15)‡
SNP8	Control	549	175 (32)	273 (50)	101 (18)	
10841	Severity ≤ grade 2	163	41 (25)	77 (47)	45 (28)	
C/A	Severity ≥ grade 3	116	26 (22)	54 (47)	36 (31)	.0042§ 1.56 (1.17-2.07)§
	IgE < 1106 IU/mL	221	57 (26)	102 (46)	62 (28)	
	IgE ≥ 1106 IU/mL	75	11 (15)	42 (56)	22 (29)	.0024 1.76 (1.25-2.49)

*Total IgE ≥ 1106 IU/mL (=75th percentile of adult asthma patient's value).
†P value corrected with Bonferroni correction (raw P values were multiplied by 2).
‡Childhood atopic asthma with IgE < 1106 IU/mL vs with IgE ≥ 1106 IU/mL (allele1 vs allele2).
§Childhood atopic asthma with severity ≥ grade 3 vs control (allele1 vs allele2).
||Childhood atopic asthma with IgE ≥ 1106 IU/mL vs control (allele1 vs allele2).

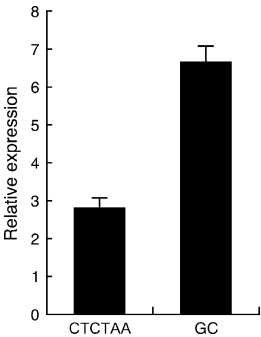


FIG 2. Effect of CTCTAA>GC polymorphism on the transcriptional activity of human *IL-12B* promoter. Relative activity was calculated by taking the relative luciferase activity of the empty vector (pGL3-basic) to be 1.

childhood atopic asthma (OR, 1.75; 95% CI, 1.22-2.51) compared with other diplotypes.

We next conducted case-control studies of patients stratified by asthma-related phenotypes, asthma severity, and high total IgE level. Association between SNP8 10841C>A and asthma was evident in childhood atopic asthma with severity ≥ 3 ($P = .0021$; corrected $P = .0042$) or with high total IgE ≥ 1106 IU/mL ($P = .0012$; corrected $P = .0024$; Table VI). We also investigated associations between asthma-related phenotypes and SNPs within patients with asthma (case-only association study), and Table VI includes the results of the case-only study. SNP1 –6415 CTCTAA>GC was associated with high total IgE ($P = .0099$; corrected $P = .020$; Table VI). A recent report showed that patients who were heterozygous for the SNP1 had increased asthma severity. In this study, the proportion of subjects with heterozygous genotypes in severe groups (severity ≥ 3) was not significantly different from those found in the groups with milder asthma (severity ≤ 2).

We examined transient expression of the CTCTAA and GC luciferase reporter constructs. Polymorphism in promoter of *IL-12B* affected relative luciferase activity

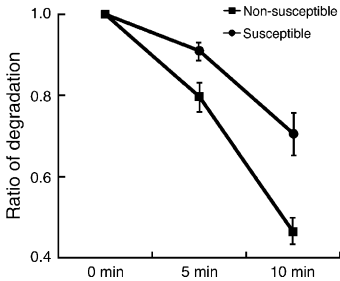


FIG 3. Stability of susceptible (3' UTR variant 10841A) and non-susceptible (10841C) transcripts of *IL-12B* mRNA measured as degradation rate. Values represent means ± SDs of data from triplicate experiments.

($P = .00018$ for comparison between allele CTCTAA and allele GC by Student t test; 2.82 [SD, 0.27] vs 6.66 [SD, 0.42], respectively; Fig 2). The GC construct had 2.4-fold higher luciferase reporter activity than the CTCTAA construct. These results suggested that the GC allele might affect the increased transcriptional activity of the *IL-12B* gene *in vivo*.

We further examined whether 3' UTR polymorphism in the coding region affected the stability of *IL-12B* mRNA. RNAs from susceptible alleles were more stable than those of the nonsusceptible alleles, and differences were significant after 5 minutes and 10 minutes of reaction time ($P = .0089$ and $.0024$, respectively; Fig 3). The current results suggested that 3' UTR polymorphism in mRNA contributed to mRNA stability.

DISCUSSION

We showed here a significant association between asthma susceptibility and an SNP in the 3' UTR region, 10841C>A, and promoter polymorphism –6415 CTCTAA>GC was also associated with asthma. Furthermore, we found a haplotype that affected the stability of transcripts and

enhanced the transcriptional level of *IL-12B*. Although the functional effects of these 2 polymorphisms were analyzed in this study, the polymorphisms that are in linkage disequilibrium with these 2 variants were not examined. -6110C>T, 1521A>G, 1645A>G, 3778G>A, and 10559WT>delG might affect the expression level of the *IL-12B* gene. Functional involvement of these linked polymorphisms in the pathogenesis of asthma should be investigated.

Several studies have surveyed the *IL-12B* gene as a potential candidate gene for asthma.¹⁹⁻²¹ Khoo et al²¹ reported that *IL-12B* promoter polymorphism was not associated with asthma susceptibility, severity, or atopy at ages 7 and 42 years in an Australian population.²¹ However, total serum IgE levels of adult men (age 42 years) with at least 1 CTCTAA allele were higher than those homozygous for the GC allele, whereas no difference was observed for adult women (geometric mean IgE = 133 kU/L vs 80 kU/L; $P = .042$). In this study, we examined childhood atopic asthma, and the mean of $\log_{10}$ (tIgE [IU/mL]) of patients was 2.69 ($= \log_{10}[490 \text{ IU/mL}]$), which was higher than the values in the Australian adult population. In the case-only study, the promoter variant was associated with high total IgE. Heterozygosity for an *IL-12B* promoter polymorphism was associated with asthma severity in a cohort of Australian children.¹⁹ Khoo et al²¹ presented no evidence to support the presence of a heterozygote effect of the *IL-12B* promoter variant on the level of asthma in early childhood and adulthood.²¹ Although we could not reproduce the finding that patients who were heterozygous for the *IL-12B* promoter polymorphism had increased asthma severity, we confirmed the association between the *IL-12B* promoter variant and asthma susceptibility and elevated serum IgE levels. Polymorphisms in *IL-12B* were found to be associated with children with asthma and atopy phenotypes in whites in the Childhood Asthma Management Program cohort using a family-based association test, and there was a strong association between the *IL-12B* 4237 and *IL-12B* 6402 polymorphisms and the asthma severity phenotype in white subjects.²⁰ The investigation confirmed positive associations for replication in a case-control study comparing adults with moderate-to-severe asthma with controls. Although we screened all 8 exons, including a minimum of 100 bases of the flanking intronic sequence of the *IL-12B* gene, we could not identify these 2 related variants reported for white subjects, *IL-12B* 4237 in intron 2 and *IL-12B* 6402 in intron 4. Although it is unclear whether these 2 polymorphisms are in LD with SNP8 in 3' UTR, we have found that variation of the *IL-12B* is associated with susceptibility of childhood atopic asthma, the asthma severity phenotype, and elevated serum IgE levels across ethnic lines.

Failure to replicate genetic associations in complex disease is a common occurrence.^{32,33} In the Japanese population, no associations were noted for 3' UTR polymorphism with asthma and allergic rhinitis using transmission disequilibrium test analyses.²⁴ They reported that preferential transmission of 10841A/C allele to

the asthma was not observed (transmitted: not transmitted = 61:60; $P > .1$). Sample size might effect on the contradictory result. Another possible explanation for the discordance with our study is unrecognized differences in environmental exposures. A recent study showed that genetic variation in *TLR2* is a major determinant of the susceptibility to asthma and allergies in a farming environment.³⁴ The functional role of genetic polymorphisms of *IL-12B*, involved in immune responses against environmental antigens, might be affected by the proportion of microbes. Furthermore, epistatic interactions may reflect the interethnic contradictory result.

IL-12 p40 encoded by *IL-12B* is a component of 4 secreted proteins that have diverse biologic function: IL-12, IL-23, p80, and the p40 monomer.⁵⁻¹⁰ It is possible for functional polymorphisms of *IL-12B* to affect production of these proteins in various tissues. Recently, functions of p80 (a p40 homodimer) and the p40 monomer in T_H2 -type immune responses were intensively studied.⁶⁻⁹ Human airway epithelial cells express programmed immune response genes, and IL-12 p40 overexpression has been observed selectively in airway epithelial cells in subjects with asthma.¹³ Our data were consistent with immunological data indicating IL-12 p40 overexpression selectivity in airway epithelial cells in subjects with asthma. Overexpression of IL-12 p40 is also an important contributing factor for the generation of the dominant T_H2 -type environment in large intestinal T_H2 -type allergic diarrhea in mice.¹² In IL-12 p40 transgenic mice, p40 functions as an IL-12 antagonist *in vivo*, and T_H1 responses in p40 transgenic mice are significantly reduced.¹¹ These findings suggest that IL-12 p40 plays a crucial role in the T_H2 immune responses as a monomer or a homodimer. We have identified a susceptibility haplotype, which effects a more stable transcript and higher levels of transcription. Although it is unclear whether the related haplotype regulates IL-12 p40 containing protein synthesis in bronchial tissue, the haplotype might contribute to the T_H2 -polarized immunity in asthma through higher IL-12 p40 monomer or homodimer production in childhood atopic asthma.

Our data strongly support the important role of *IL-12B* in asthma. Further investigations of the connection between genotypes and the functional role of IL-12 p40 would be helpful to clarify the etiology of asthma.

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