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Amino-acid substitutions in the *IKAP* gene product significantly increase risk for bronchial asthma in children

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Abstract The complex etiology of bronchial asthma (BA), one of the most common inflammatory diseases throughout the world, involves a combination of various genetic and environmental factors. A number of investigators have undertaken linkage and association studies to shed light on the genetic background of BA, but the genetic aspects of this disease are still poorly understood. In the course of a project to screen the entire human genome for single nucleotide polymorphisms (SNPs) that might represent useful markers for large-scale association analyses of common diseases and pharmacogenetic traits, we identified six SNPs within the gene encoding I-κB-associated protein (IKAP), a regulator of the NF-κB signal pathway. Most of these SNPs were in linkage disequilibrium with each other. We observed a strong allelic association between BA in childhood and two of the SNP sites, T3214A (Cys1072Ser) and C3473T (Pro1158Leu); $P = 0.000004$ for T3214A and $P = 0.0009$ for C3473T. T3214A was also associated with BA in adult patients ($P = 0.000002$), but C3473T was not ($P = 0.056$). To confirm the above results, we compared estimated frequencies of haplotypes of the six SNPs between BA patients and controls. We found a strong association between BA in childhood and a specific haplotype, TGAAAT, that involved two amino-acid substitutions (819T, 2295G, 2446A, 2490A, 3214A, and 3473T; $P = 0.00004$, odds ratio, 2.94; 95% confidence interval [CI], 2.48–3.4). On the other hand, haplotype TACGTC, which differed from the TGAAAT haplotype in the last five nucleotides, was inversely correlated with the BA phenotype ($P = 0.002$; odds ratio, 9.83; 95% CI, 8.35–11.31). These results indicated that specific variants of the *IKAP* gene, or a variant in linkage disequilibrium with the TGAAAT haplotype, might be associated with mechanisms responsible for early-onset BA.

Key words Bronchial asthma · SNP · IKAP · Haplotype · Association study

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Introduction

Bronchial asthma (BA), a disease characterized clinically by chronic, intermittent airway obstruction, with wheezing, coughing, and breathlessness, now affects 3% of adults and 6% of children in Japan. This complex disorder is considered to reflect a combination of various genetic and environmental factors. Many linkage and association studies have been performed with polymorphic DNA markers to disclose genetic components involved in the pathogenesis of BA. Manian (1997) reviewed studies showing linkage of atopy to chromosome 11, and linkage of atopy and bronchial hyperresponsiveness to chromosomes 5q and 12q. Others have suggested associations also between the BA phenotype and polymorphisms in the β2 adrenoceptor (Hopes et al. 1998), tumor necrosis factor (TNF; Moffatt and Cookson 1997), platelet-activating factor (Stafforini et al. 1999), granulocyte/macrophage-colony stimulating fac-

tor (Rohrbach et al. 1999), and interleukin 4 receptor (Ober et al. 2000), among others.

Genome-wide screenings for susceptibilities to asthma and atopy have highlighted several additional candidate regions (Manian et al. 1997). One such locus is at chromosome 9q, where Wjst et al. (1999) have mapped a locus linked to BA susceptibility ($P = 0.0073$), elevated serum immunogloblin E (IgE; $P = 0.0098$), and positive radioallergosorbent tests (RAST; $P = 0.0025$). Their study involved 97 German and Swedish families and included 415 persons and 156 sib pairs. The gene encoding IKK complex-associated protein (*IKAP*), which lies at 9q34, was considered to be a candidate for BA on positional grounds.

Single-nucleotide polymorphisms (SNPs), the most frequent type of genetic variation in the human genome, represent useful markers for association studies of common diseases and pharmacogenetic traits (Brookes 1999, Cargill et al. 1999, Evans and Relling 1999). To investigate possible associations of alleles at SNP sites with complex diseases such as BA, ischemic heart disease, and rheumatoid arthritis, we recently started a program to screen SNPs on a genome-wide scale (Ohnishi et al. 2000, Unoki et al. 2000, Yamada et al. 2000), bearing in mind that haplotype analysis of multiple SNPs is especially powerful for investigating alleles responsible for genetically complex diseases (Stephens et al. 1998, Tishkoff et al. 2000). Indeed, disorders such as Alzheimer disease and hypertension have been intensively analyzed in this way already (Jeunemaitre et al. 1997, Martin et al. 2000).

In the study reported here, we performed extensive screening of *IKAP* by direct sequencing to detect alleles at polymorphic sites that might be responsible for BA. We identified six distinct SNPs in the coding region of the *IKAP* gene, and identified allelic associations between BA and these SNPs. Further, we defined an association between a specific six-SNP haplotype and BA of childhood onset.

Methods

Subjects

Peripheral blood was obtained, with written informed consent, from each of 235 pediatric BA outpatients at the Osaka Prefectural Habikino Hospital (mean age, 9.57 years; range, 1–17 years; male/female ratio, 1.47:1.0; mean serum IgE level, 1005.9 U/ml). Most of these children had been diagnosed with atopic asthma. A total of 270 adult BA patients were also studied, 103 from the Internal Medicine Unit at the Osaka Prefectural Habikino Hospital and 167 from the Miyatake Asthma Clinic (mean age, 45.9 years; range, 18 to 83 years; male/female ratio, 1.0:1.16). Of the adult patients, 20% were atopic, but serum IgE levels were not available. As controls we analyzed 372 randomly selected, population-based individuals. Adult BA patients who reported episodes of asthma in childhood were excluded from the study. All patients had been diagnosed with BA according to the guidelines of the Japanese Society of Allergology, and disease status was classified into four categories: (1) no medication, (2) sodium chromoglycate (DSCG) and/or theophyline, (3) inhaled glucocorticoid, less than 400 μg/day; and (4) inhaled glucocorticoid, more than 400 μg/day. cDNAs were obtained from 51 volunteer individuals according to standard protocols, and genomic DNAs were prepared from all participants according to standard protocols. SNPs were screened according to methods we described previously (Ohnishi et al. 2000). This study was approved by the Institutional Review Board (IRB) of the Institute of Medical Science, The University of Tokyo.

Screening for polymorphisms

Eight primer sets (Table 1) were designed on the basis of the *IKAP* cDNA sequence available from GenBank (accession number, AF044195), so as to screen the entire coding region. Each polymerase chain reaction (PCR) was performed with 40 ng of mixed cDNA from three individuals. Reaction volumes of 50 μl contained dNTPs (25 mM), MgCl2 (6.68 mM), 16.6 mM NH4SO4, 6.7 mM TrisHCl (pH 8.8), 10 mM β-mercaptoethanol, two sets of primers (F1 and R4, and F4 and R8, 50 pmol each), and *Ex-Taq* (2.5 U; TaKaRa, Otsu, Japan). Samples were amplified in the GeneAmp PCR system 9600 (PE Applied Biosystems, Norwalk, CT, USA). Thermal-cycling conditions were 94°C for 2 min; then 36 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 30 s, with final extension at 72°C for 10 min. Then each PCR product was reacted with BigDyeTM Terminator RR mix (PE Applied Biosystems) and one internal primer from among the 16 listed in Table 1. Sequences obtained in an ABI Prism 377 DNA autosequencer (PE Applied Biosystems) were assembled, and polymorphisms were identified by the Polyphred program (Nickerson et al. 1997).

Table 1. Primers used in screening for SNPs

F1	GTACTGTTCTCGTGGGAATCACTT	**R1**	CTTCTGATCCATGGAACTGTGTC
F2	ACTGTTGGATGGGGTAGGAAG	**R2**	CCACAGGTGCTGAAGGATAAACT
F3	GGAAACTATCACTGGTATCTCAAGC	**R3**	ATCCAAGTGAGAAGGCCTAGTTT
F4	TGAAGATCAAGATGTAAACCCG	**R4**	CCGGCCTGTAATGTTTTAAATG
F5	CCATACCTGCCAGTGTTTTTG	**R5**	CATGAGATGTAAGTATGGATAGGCA
F6	GGATCCTGACGGGAATAAAATAG	**R6**	ACATGTGAGAAAGGCTGAGAGAG
F7	ACAACAGTACCAGGATATCAGCA	**R7**	GGAGTATTTGCCACTCATCTCAC
F8	GCAGGTCTGGATGATGAGGTA	**R8**	TGAGTGGAAATGGTCTTCTCTGT

SNP, Single nucleotide polymorphism

Table 2. PCR conditions and oligomers used for ASO hybridization

SNP	Forward primer	Reverse primer	PCR conditions (°C)		Allele-specific oligonucleotides	Conditions for hybridization	
			Annealing	Extension		°C	h
C819T	CAGGCAGTTTGATTGCATCTA	CTCATCTTTAAGGAAGGGAAG	56	72	TGGACT(C/T)CTTCATG	37	4
G2295A	AAACTAATATGATCTGAACGAAG	AAACTATGGAAAAAGATACACATG	53	72	CACATT(C/T)CCAAGAAAC	37	4
A2446C	CCAGTTACCAGCAGTGTCCA	TTTCCAGTTCTGGGGTTGTC	57	72	GAATAAA(A/C)TAGACCT	37	4
A2490G	CCAGTTACCAGCAGTGTCCA	TTTCCAGTTCTGGGGTTGTC	57	72	AGAGCAT(A/G)AATCCTC	37	4
T3214A	CAGGAAAGCTGGTTGAGCAG	CAATTGAGAAGACCTTGCTTG	57	72	GAAGAG(A/T)GTGCCC	37	4
C3473T	GTAGTTCGAGAGCTCAAGGA	TGCCACTCACGACAC TGCT	57	72	GGTAC(C/T)CCACGG	37	4

PCR, Polymerase chain reaction; ASO, allele-specific oligonucleotide

Amplification and genotyping of samples

All genomic DNA samples were amplified in the GeneAmp PCR system 9600 (PE Applied Biosystems), using primers designed so that each PCR product would include one or two SNPs. Conditions for thermal cycling and hybridization appear in Table 2. The PCR products were dot-blotted and fixed by UV-crosslinking on Biodyne membranes (PALL; Northern Boulevard East Hills, NY, USA) according to the manufacturer's protocol, then hybridized with ^{32}P-labeled allele-specific oligonucleotide (ASO) sequences. For the C3473T locus, restriction fragment length polymorphism (RFLP) analysis/PCR was performed on the same materials to confirm the ASO results. Each reaction mixture contained 10 μl of PCR product, 3 U of *Nla*IV (1 U/μl; BioLabs; Beverly, MA, USA), 2 μl of 10x Buffer K (TaKaRa), and 15 μl of 0.01% bovine serum albumin (BSA; TaKaRa). After 1 h of incubation at 37°C, the products were electrophoresed on 2% agarose gels.

Statistical analysis

Allele frequencies in BA cases and controls were compared by the contingency χ^2 test, using StatView-J 4.02 software (Abacus Concepts, Berkeley, CA, USA). Odds ratios were estimated according to Brown (1981). Phase-unknown samples, including 207 from controls, 235 from children with BA, and 90 from adult BA patients, were subjected to haplotype analysis, based on a maximum-likelihood method, using ARLEQUIN software Ver.2.0 (Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Geneva, Switzerland). Haplotype frequencies between cases and controls were evaluated by χ^2 tests in which the test of homogeneity was made with one degree of freedom rather than a global test with multiple degrees of freedom. Instead, probability values were corrected for multiple comparisons by multiplying the *P* value by the number of haplotypes compared (Bonferroni adjustment). In addition, pairwise linkage disequilibrium coefficients were calculated and expressed as the $D' = D/Dmax$ or $D/Dmin$, according to Thompson et al. (1988).

Results

We screened the *IKAP* gene for genetic variations among 51 Japanese subjects and identified six SNPs within the coding region: T819C (Leu273Leu), G2295A (Gly765Gly), A2446C (Ile816Leu), A2490G (Ile830Met), T3214A (Cys1072Ser) and C3473T (Pro1158Leu). Their locations are shown in Fig. 1. Leu273Leu is a silent substitution located in the WD-like repeats. Ile244Leu and Ile2490Met are in the IKKα binding site, and Cys3214Ser is in IKKα/IKKβ binding sites; Pro3473Leu lies in the serine-rich domain. Although two of these polymorphisms are silent changes, the four nonsynonymous substitutions would create missense alterations in the protein product.

Subsequently we performed an association study using these SNPs against the BA phenotype. All six of these loci

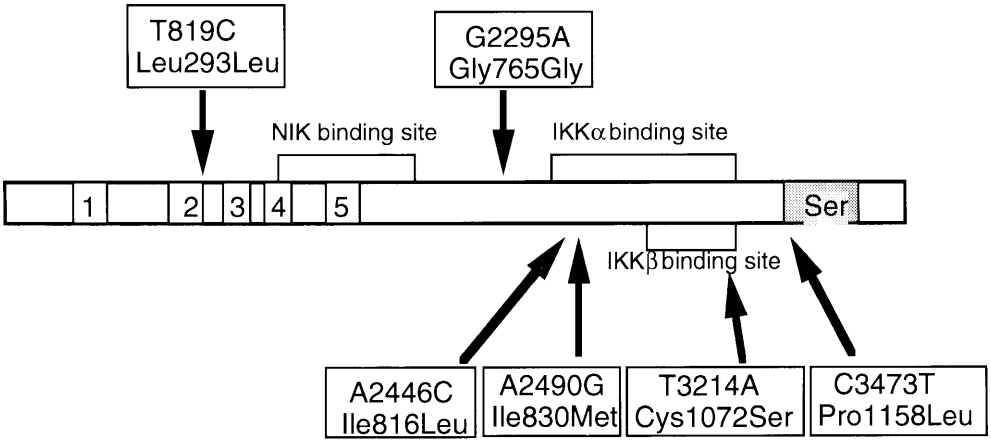

Fig. 1. Locations of six single nucleotide polymorphisms (SNPs) in the *IKAP* gene. Five WD-like repeats are numbered, and binding sites for NIK and two IKKs are indicated. The SNPs labeled *above the bar* are synonymous; those *below the bar* would cause missense substitutions in the protein. The bronchial asthma (BA)-associated T3214A polymorphism lies in the IKKα and IKKβ binding domain, and the C3473T polymorphism lies within the region encoding a serine-rich domain. *Leu*, Leucine; *Gly*, glycine; *Ser*, serine; *Ile*, isoleucine; *Met*, methionine; *Pro*, proline; *NIK*, nuclear factor-κB-inducing kinase, *IKK*; I-κB kinase

were in Hardy-Weinberg equilibrium in the control group. When pairwise linkage disequilibrium was examined between the *IKAP* SNPs (Table 3), most of the SNPs were in linkage disequilibrium with each other. In particular, G2295A-A2490G and T3214A-C3473T showed quasi-complete linkage disequilibrium ($D' = 0.966$, $\chi^2 = 124.0$ and $D' = 0.884$, $\chi^2 = 256.1$). The BA patients were divided into two groups according to age of onset (younger than 18, or 18 years and older); the younger than 18 years group (BA in childhood) represented atopic BA, and the over-18 group was rich in non-atopic BA. The six SNPs were examined in 235 pediatric BA samples and 266 adult BA samples, together with 294 controls, for comparisons of allelic frequencies (Table 4). In childhood BA we observed strong allelic associations with T3214A and C3473T ($\chi^2 = 16.29$, df = 1, $P = 0.000004$ for T3214A, and $\chi^2 = 11.09$, df = 1, $P = 0.0009$ for C3473T). As already shown in Table 3, these two sites are associated strongly with each other. The T3214A allele was also associated with adult-onset BA ($\chi^2 = 21.88$, df = 1, $P = 0.000002$), but C3473T was not ($\chi^2 = 3.644$, df = 1, $P = 0.056$). The other four SNPs showed no association with BA, either in children or adults. When BA patients were further subdivided by severity according to the guidelines of the Japanese Society of Allergology, these SNPs failed to detect any differences regarding clinical severity (data not shown). These results indicated that Ser at codon 1072 and Leu at codon 1158 of the *IKAP* gene, or another genetic variant in strong association with these two substitutions, may have an important role in the etiology of BA, particularly BA of early onset.

Estimated frequencies of the six-locus haplotypes were compared between BA in childhood and controls, and between adult-onset BA patients and controls (Table 5). Although 26 different haplotypes were observed in the test population, 9 predominant haplotypes (frequency, more than 1% in controls) reflected linkage disequilibrium. Because the 17 minor haplotypes were combined as "others" (see Table 5), the estimated *P* values were adjusted by multiplying the number of haplotypes tested (Bonferroni adjustment). The most common haplotype was 819T, 2295G, 2446A, 2490A, 3214T, and 3473C (TGAATC) in all groups. A strong association with BA in childhood was observed with a TGAAAT haplotype ($\chi^2 = 22.34$; df, 1; $P = 0.00004$; odds ratio, 2.94; 95% CI, 2.48–3.4; Table 5), in which two amino-acid substitutions were present (1072 from Cys to Ser and 1158 from Pro to Leu). However, a TACGTC haplotype, in which the last five nucleotides differed from the TGAAAT haplotype, was very uncommon in BA in childhood and was inversely correlated with the BA phenotype ($\chi^2 = 13.65$; df, 1; $P = 0.002$; odds ratio, 9.83; 95% CI, 8.35–11.31). The relative risk for BA among individuals bearing the TGAAAT haplotype is therefore nearly 25 times higher than that for those individuals carrying the TACGTC haplotype ($\chi^2 = 23.38$; df, 1; odds ratio, 25.12; 95% CI, 23.56–26.68).

Table 3. Pairwise linkage-disequilibrium coefficients between six SNPs in control samples

	D′				
Polymorphism	T819C	G2295A	A2446C	A2490G	T3214A
G2295A	0.403	—	—	—	—
A2446C	0.308	0.735	—	—	—
A2490G	0.485	0.966	0.696	—	—
T3214A	0.103	0.344	0.33	−0.943	—
C3473T	0.14	0.371	0.341	−0.686	0.884

Disequilibrium is expressed as D′/Dmax or Dmin, according to Thompson et al. (1988)

Discussion

Currently available techniques have been unveiling the molecular etiologies of many human diseases at a rapid pace, especially those of monogenic origin. However, eluci-

Table 4. Result of genotyping on the six SNPs

	Control	Percentage	Childhood BA	Percentage	χ^2 (df = 1)	*P*	Adult BA	Percentage	χ^2 (df = 1)	*P*
Allele	*n*		*n*				*n*			
T819C (Leu273Leu)										
T	553	82.5	376	80	1.179	0.277	425	83.7	0.259	0.618
C	117	17.5	94	20			83	16.3		
Total	670		470				508			
G2295A (Gly765Gly)										
G	374	68.8	339	72.1	1.378	0.24	317	70.4	0.334	0.563
A	170	31.2	131	27.9			133	29.6		
Total	544		470				450			
A2446C (Ile816Leu)										
A	466	73.7	337	71.7	0.563	0.453	353	93.4	0.002	0.965
C	166	26.3	133	28.3			125	6.6		
Total	632		470				378			
A2490G (Ile830Met)										
A	550	87	417	88.7	0.723	0.395	458	88.4	0.509	0.475
G	82	13	53	11.3			60	11.6		
Total	632		470				518			
T3214A (Cys1072Ser)										
T	438	74.5	296	63	16.291	0.000004	327	61.5	21.882	0.000002
A	150	25.5	174	37			205	38.5		
Total	588		470				532			
C3473T (Pro1158Leu)										
C	529	71.1	291	61.9	11.091	0.0009	357	66.1	3.644	0.056
T	215	28.9	179	38.1			183	33.9		
Total	744		470				540			

BA, Bronchial asthma; df, degree of freedom

dation of the genetic basis of common diseases such as essential hypertension, diabetes mellitus, obesity, and asthma, is a major challenge in human genetics because their multifactorial etiologies involve both genetic and environmental factors. Among diseases of this type, bronchial asthma (BA) is probably the condition most extensively investigated so far. Genome-wide linkage studies of atopic asthma performed by numerous investigators in a variety of populations have detected several candidate regions, and allelic-association studies have been undertaken with candidate genes, such as β2 adrenoceptor (Hopes et al. 1998), tumor necrosis factor (TNF) (Li Kam Wa et al. 1999), platelet-activating factor (Stafforini et al. 1999), granulocyte/macrophage-colony stimulating factor (Rohrbach et al. 1999), and interleukin 4 receptor (Ober et al. 2000). Another locus implicated in BA lies on chromosome 9q (Wjst et al. 1999).

Transcription factors belonging to the nuclear factor-κB (NF-κB) family are critical regulators of genes involved in inflammation, cell proliferation, and apoptosis. NF-κB is inactivated by binding to I-κB, and activated through phosphorylation of I-κB. IKAP (IKK complex-associated protein) is a scaffold protein of the I-κB kinase complex that consists of NF-κB-inducing kinase (NIK), I-κB kinase (IKK)-α, and IKK-β. Therefore IKAP is regarded as a regulator of at least three different kinases involved in the pro-inflammatory NF-κB signaling pathway (Cohen et al. 1998, Mercurio et al. 1999), and it seemed to be a likely candidate for association with BA. The *IKAP* gene lies on chromosome 9q34, where Wjst et al. (1999) mapped a locus linked to BA susceptibility ($P = 0.0073$), elevated serum immunogloblin E (IgE; $P = 0.0098$), and positive radioallergosorbent tests (RAST; $P = 0.0025$) using DNA samples from 97 German and Swedish families that included 415 persons and 156 sib pairs. Interleukin 1 (IL-1), TNFα, leukotriene B4, and other physical-stress factors, such as exposure to allergens, can activate IKKs; in that case, NF-κB signaling via I-κB stimulates the production of pro-inflammatory mediators such as IL-1β, IL-2, IL-6, IL-8, and IL-12. All these molecules are likely to be involved in the inflammatory processes of asthma. On the basis of mapping information and the other cited evidence, we assumed that *IKAP* would be a likely candidate for some important role in the pathogenesis of BA.

We genotyped BA patients and control individuals for the six SNP sites present in the coding region of the *IKAP* gene, and detected a strong allelic association between BA in childhood and T3214A (Cys1072Ser) and C3473T (Pro1158Leu) polymorphisms ($P = 0.000004$ for T3214A and $P = 0.0009$ for C3473T as compared with controls). A strong association was also observed between adult-onset BA and T3212A, but not C3473T. The evidence of allelic association suggests an etiological role of *IKAP* in BA, especially BA of early onset. BA in childhood is often associated with atopy and allergenic load derived from house dust, mites, and pollen, and about 90% of child/juvenile patients have elevated serum IgE levels (Woolcock and Peat, 1997). Some genetic and environmental aspects, as they relate to differences in adult or childhood forms of BA, have been recorded elsewhere (Hopes et al. 1998). The juvenile BA patients who participated in this study presented elevated serum IgE and almost all cases were diagnosed as atopic asthma. Most of the adult BA patients recruited for the present study were nonatopic, because we

Table 5. Frequencies of six-locus haplotypes and odds ratios in the control group and in patients with BA in childhood or adult onset

Locus	(Analyzed allele number) Haplotype					Control (372) Freq	Childhood BA (470) Freq	Adult BA (180) Freq	Control vs childhood BA				Control vs adult BA			
									χ^2 (df = 1)	P*	Odds ratio	95% CI	χ^2 (df = 1)	P*	Odds ratio	95% CI
	819	2295	2446	2490	3214	3473										
	T	G	A	A	T	C	0.498	0.413	0.406	6	0.143	1.41	(1.14–1.68)	4.1	0.707	1.45	(1.09–1.81)
	T	G	A	A	A	T	0.070	0.182	0.135	22.34	0.00004	2.94	(2.48–3.4)	5.93	0.149	2.05	(1.46–2.64)
	T	A	C	A	A	T	0.092	0.131	0.141	3.37	0.662	1.51	(1.07–1.95)	2.87	0.905	1.6	(1.05–2.15)
	C	A	C	G	T	C	0.042	0.091	0.057	7.49	0.062	2.24	(1.65–2.83)	0.43	>1	1.31	(0.5–2.12)
	C	G	A	A	T	C	0.024	0.063	0.078	7.39	0.066	2.75	(1.99–3.51)	8.72	0.031	3.4	(2.54–4.26)
	T	A	C	G	T	C	0.039	0.004	0.034	13.65	0.002	9.83	(8.35–11.31)	0.16	>1	1.22	(0.26–2.18)
	T	A	A	A	A	T	0.030	0.004	0.006	8.75	0.031	7.13	(5.62–8.64)	3.29	0.697	5.45	(3.4–7.5)
	C	G	A	A	A	T	0.033	0.015	0.018	3.6	0.578	2.4	(1.47–3.33)	1.44	>1	2.4	(0.87–3.41)
	C	A	C	A	A	T	0.014	0.016	0.025	0.03	>1	1.11	(0.05–2.27)	0.58	>1	1.67	(0.34–3)
	Others						0.158	0.081	0.100								
						Total	1.000	1.000	1.000								

Bonferroni-type adjustment is corrected with ×10; the nine predominant haplotypes are listed; the "others" category includes 17 minor haplotypes (<1% frequency)
BA, Bronchial asthma; *P** Corrected with Bonferroni-type adjustment; CI, Confidence interval

excluded patients who reported episodes of BA in childhood. Because we observed a positive association between adult-onset BA and the T3214A allele but not C3473T, probably an admixture of atopic and nonatopic phenotypes was present in this group.

Because the polymorphisms identified here as associated with BA in childhood occurred within the coding region of *IKAP* and substituted Cys for Ser in the IKKs binding site and/or Leu for Pro in the serine-rich domain of the protein, it is possible that these alterations, singly or together, affect the signaling pathway by modifying the activity of *IKAP*. Alternatively, base substitution(s) elsewhere in this gene, i.e., in the promoter region or in introns, might account for susceptibility to BA by affecting the quality or quantity of the *IKAP* product, or another genetic variant in nearby gene(s) in strong linkage disequilibrium with these substitutions might have an important role in the etiology of BA.

When we combined multiple SNP sites and analyzed haplotypes, we observed only nine predominant haplotypes, because all six SNPs in the *IKAP* gene were in linkage disequilibrium with each other to various degrees (Table 5). We detected significant associations of the haplotype TGAAAT (odds ratio, 2.94; 95% CI, 2.48–3.4) and haplotype TACGTC (odds ratio, 9.83; 95% CI, 8.35–11.31), with BA in childhood-onset BA but not in the adult form. Because the TACGTC haplotype is more frequently present in controls, it could be interpreted as a protective or resistant allele. Therefore the relative risk of BA for individuals bearing haplotype TGAAAT is nearly 25 times higher than that for those individuals carrying haplotype TACGTC (χ^2 = 23.38; df, 1; odds ratio, 25.12; 95% CI, 23.56–26.68). Because the TGAAAT haplotype is associated with two amino-acid substitutions, i.e., in the IKK-β binding domain and in the serine-rich domain of *IKAP*, an allele with this haplotype might alter the role of IKAP as a scaffold protein, accelerating or decelerating the signaling of NF-κB. To fully understand the effects of *IKAP* SNPs on the asthma phenotype, especially in childhood, it may be desirable in future studies to investigate the relationship between genotypes and the functional role of the SNPs in this gene.

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