

Association of atopy and eczema with polymorphisms in T-cell immunoglobulin domain and mucin domain—IL-2-inducible T-cell kinase gene cluster in chromosome 5q33

Penelope E. Graves, ScD,* Valérie Siroux, PhD,* Stefano Guerra, MD, PhD,
Walter T. Klimecki, DVM, PhD, and Fernando D. Martinez, MD Tucson, Ariz

Background: The T-cell immunoglobulin domain and mucin domain (*TIM*) gene family and the gene for IL-2-inducible T-cell kinase (*ITK*), located in chromosome 5q33 and potentially involved in the T-cell proliferation and differentiation, are good candidate genes for allergic diseases. **Objective:** We assessed the role of polymorphisms in the *TIM* family genes and *ITK* in atopy, eczema, and asthma. **Methods:** Twenty-one polymorphisms in the *TIM-ITK* gene cluster were genotyped in 564 children enrolled in the Tucson Children's Respiratory Study. Skin prick tests to common allergens were performed at age 6.1 years (n = 508), age 10.8 years (n = 539), and age 16.6 years (n = 424). Asthma and eczema were assessed by questionnaire at these 3 points. Averaged relative risks were estimated.

Results: One 15-bp insertion/deletion in exon 4 of *TIM1* was significantly related to atopy and eczema (relative risk associated with carrying at least 1 rare allele = 1.24 [1.07-1.45], $P = .005$; and 1.43 [1.01-2.01], $P = .004$, respectively). The 3 tested single nucleotide polymorphisms (SNPs) in *TIM3* were significantly related to atopy and eczema. One of them, at position +4259 calculated from the translation start site, predicts a putative change in the amino acid sequence of the protein, and was the most strongly related to atopy (relative risk = 1.28 [1.12-1.47]; $P = .0003$). SNPs in the 5' genomic region in *ITK*, which show moderate linkage disequilibrium with those in *TIM3*, had an independent effect on atopy. None of the polymorphisms studied was related to asthma.

Conclusion: Our findings support a potential role for SNPs in *TIM1*, *TIM3*, and *ITK*, independent of each other, in allergic diseases. (J Allergy Clin Immunol 2005;116:650-6.)

Key words: Atopy, asthma, eczema, *TIM*, *ITK*, polymorphisms

Abbreviations used

CRSP9: cofactor required for Sp1 transcriptional activation, subunit 9
HAV: Hepatitis A virus
ITK: IL-2-inducible T-cell kinase
LD: Linkage disequilibrium
RR: Relative risk
SNP: Single nucleotide polymorphism
SPT: Skin prick test
TIM: T-cell immunoglobulin domain and mucin domain

There is convincing evidence suggesting that CD4⁺ lymphocytes of the T_H2 type play a pivotal role in the pathogenesis of allergic diseases. Genetic factors are known to be important determinants of the allergic phenotype, and a large number of linkage and association studies have been published and were recently reviewed.¹ The results of these studies have suggested that many genes may influence the susceptibility to T_H2-mediated conditions. This marked genetic heterogeneity may be in part a result of the important influence of environmental factors in the etiology of allergic conditions,² but it may also be attributable to the large number of gene products that regulate T_H2-mediated immune responses.

Several linkage studies have suggested that a region on chromosome 5q31-33 contains genetic variations associated with asthma and other allergic conditions.³⁻⁷ Recently, a set of genes that cluster in chromosome 5q33.3 has been shown to play a role in the immune responses that regulate the development of allergic diseases. This cluster is composed of the genes of the family of T-cell immunoglobulin domain and mucin domain (TIM) proteins (TIM1, TIM3, and TIM containing 4 [TIMD4]) and the IL-2-inducible T-cell kinase (ITK), all of which are expressed in T cells.^{8,9} McIntire et al¹⁰ identified in mice the genes for TIM1 and TIM3 and found that polymorphisms in these genes were associated with the development of airway reactivity and IL-4 production by T cells.¹⁰ The human homologue of *Tim1* is the cellular receptor for the hepatitis A virus cellular receptor 1 (HAVCR-1) or *TIM1*,¹¹ and a study suggested a potential role of genetic variation in this gene in allergic disease, in association with hepatitis A infection.¹² This association

From the Arizona Respiratory Center.

*These authors contributed equally to this work.

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Reprint requests: Penelope E. Graves, ScD, Arizona Respiratory Center, University of Arizona, 1501 N Campbell Avenue, PO 245030, Tucson, AZ 85724-5030. E-mail: pgraves@u.arizona.edu.

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of a *TIM1* polymorphism with atopy has been replicated in some but not all populations.^{13,14} *TIM3* is located 28 kb telomeric from *TIM1* and is expressed in T_H1 cells.¹⁵ Abrogation of interaction between *TIM3* and its ligand in a mouse model led to heightened T_H1 responses,¹⁶ suggesting the possibility that *TIM3* may be involved in the susceptibility to T_H1-mediated and T_H2-mediated diseases.¹⁷ Finally, *ITK* is located 72 kb telomeric from *TIM3* and is known to be involved in important signaling pathways that regulate lymphocyte function.⁹ CD4⁺ T cells from *ITK*-deficient mice secrete significantly less IL-4 than wild-type T cells and exhibit ineffective T_H2 responses.^{18,19} However, paradoxically, *Itk*^{-/-} mice have increased basal serum total IgE as well as antigen specific IgE.¹⁹

To assess the potential role of polymorphisms in genes of the *TIM* family and *ITK*, we genotyped children enrolled in the Tucson Children's Respiratory Study for 21 genetic variants in *TIMD4*, *TIM1*, *TIM3*, *ITK*, and 2 other genes and gene structures located within the same cluster: a cofactor required for Sp1 transcriptional activation, subunit 9 (*CRSP9*), and *NM-130899*.

METHODS

Population

The children included in the current analysis were a subset of the 1246 healthy infants recruited in the Tucson Children's Respiratory Study. The enrollment process and study design are described elsewhere.²⁰ Briefly, unselected newborns were enrolled between 1980 and 1984. Parents planning to use a large health maintenance organization in Tucson were contacted shortly after their child's birth. Thereafter, all new siblings were recruited in the study. In the current analysis, only the first enrolled child within each family with at least 1 white or Hispanic parent was eligible ($n = 1088$). Among these 1088 subjects, DNA was available for 568 subjects.

Longitudinal study of asthma and wheezing

The purpose of the Tucson Children's Respiratory Study was to assess prospectively the risk factors for asthmalike symptoms occurring during childhood. For this purpose, multiple questionnaires ascertaining parental characteristics and the child's respiratory health were completed by the parents at different ages. Data for the current analysis were derived from the enrollment survey, the year 6 survey (6.1 ± 0.8 years), the year 11 survey (10.8 ± 0.5 years), and the year 16 survey (16.6 ± 0.6 years).

Skin prick tests

Skin prick tests (SPTs) to common allergens in Tucson were performed at the year 6, year 11, and year 16 surveys. Among the 568 eligible subjects with available DNA, 564 had SPT measurement in at least 1 survey. The allergens that were consistently tested at every survey were Bermuda grass, olive tree, careless weed, *Alternaria alternata*, mesquite tree, and mulberry tree (all allergens obtained from Hollister-Stier Laboratories, Everett, Wash). The sum of 20 perpendicular diameters was calculated for each wheal after 20 minutes. Wheal sizes 3 mm or greater than the negative control were

considered positive, and atopy was defined as a positive reaction to at least 1 allergen.

Eczema and asthma

Both eczema and asthma were ascertained by questionnaire at the year 6, year 11, and year 16 surveys. Eczema was defined as a positive answer to the question, "Has this child ever had eczema (allergic skin reaction)?" Asthma was defined as having a positive response to the questions, "Has your child ever had asthma (reactive airways disease)?" and "Did a doctor ever say this child had asthma?" In addition, parents also had to report that the child had 1 or more episodes of wheezing during the previous year. This definition has been used previously in this population.²¹

Polymorphism detection

Single nucleotide polymorphisms (SNPs) were identified in the chromosome region which includes *CRSP9*, *NM130899*, and the *ITK* promoter by denaturing high-performance liquid chromatography (DHPLC) using the WAVE DNA Fragment Analysis System (Transgenomic, Inc, Santa Clara, Calif). A description of these methods has been reported previously.²² In addition, the *ITK* gene was resequenced in 23 subjects in each of 3 ethnic groups (white, African American, and Hispanic), and the *TIM1* gene was resequenced in the same white and African American subjects. Methods used for resequencing are described in the Program for Genomic Applications Web site (innateimmunity.net). Polymorphisms in each gene with a minor allele frequency of $\geq 10\%$ were grouped on the basis of their level of linkage disequilibrium. One polymorphism from each group (and a second SNP from 1 group) was selected for genotyping. SNPs that induced a change in the amino acid sequence of the protein or were located in the promoter region were selected for genotyping when several SNPs were in linkage disequilibrium. SNPs in *TIM3* and *TIMD4* were identified in database SNP (dbSNP).

Subjects were genotyped for each SNP by PCR amplification of the region containing the polymorphism and selective restriction endonuclease digestion or template-directed dye-terminator incorporation with fluorescence polarization. Locations of all polymorphisms and reference SNP ID (rs) (if available) together with the primers and assay conditions for all assays are listed in Table E1 (see the Online Repository in the online version of this article at www.mosby.com/jaci).

Statistical analysis

The frequencies of the polymorphisms in the study population were calculated and compared with a population in Hardy-Weinberg equilibrium by using a χ^2 test. Both the r^2 and the D' statistics were used to estimate linkage disequilibrium (LD) between polymorphisms, although only r^2 is reported. Cross-sectional associations between each of the 21 polymorphisms and atopy were studied separately in the 3 surveys. The χ^2 test was used to assess statistical significance. On the basis of the longitudinal design of the study, generalized estimating equations, which take into account the intra-subject serial correlation, were used. The statistical methods have been expanded in the Online Repository (see the statistical analysis in the online version of this article at www.mosby.com/jaci). Because of the multiple comparisons involved in these analyses, P values were adjusted by using the method of Benjamini and Hochberg²³ that controls for the false discovery rate.²⁴

To assess the potential role of combinations of SNPs on the phenotypes, haplotype analyses were performed. Haplotypes were assessed by using PHASE software with a 95% level of confidence for the phase inference.^{25,26}

TABLE I. Comparison of the subset of children included in the current analysis to the children not included (without DNA sample and/or without SPT)

	Children included in the analysis	Children without DNA or SPT	P
n	564	524	
Sex, % male	49.5	48.5	.74
Education			
Maternal education	73.0	68.7	.11
at enrollment >12 y, %			
Paternal education at	71.8	63.6	.004
enrollment >12 y, %			
Ethnicity			
2 Parents white, %	60.1	62.8	<.0001
1 Parent Hispanic/1	14.4	6.5	
parent white, %			
2 Parents Hispanic, %	14.5	8.0	
Other, %	11.0	22.7	
Smoking			
Maternal smoking at	16.3	19.1	.23
enrollment, %			
Paternal smoking at	30.2	33.1	.31
enrollment, %			
Child smoking at year	55.0	46.7	.29
16, %			
Eczema			
Child eczema (at least	32.2	41.0	.03
1 survey), %			
Asthma			
Maternal history of	10.5	12.7	.28
asthma, %*			
Paternal history of	13.8	10.5	.12
asthma, %*			
Child asthma (at least 1	32.3	28.6	.34
survey), %†			

*Medically diagnosed asthma.

†Medically diagnosed asthma and at least 1 wheezing episode in the past year.

RESULTS

Genotyped children who underwent SPT on at least 1 survey (n = 564) were compared with the children enrolled in the study but who were not included in the current analysis mainly because DNA was not available (n = 524). Participants were more likely to have a father with a higher level of education, and Hispanic parents and were less likely to report eczema than nonparticipants (Table I).

The prevalence of atopy was 39.4%, 54.6%, and 71.2% for year 6 (n = 508), year 11 (n = 539), and year 16 (n = 424) surveys, respectively. The frequency of atopy did not differ according to ethnicity at any survey. Frequency of eczema was 15.4%, 17.6%, and 17.8%, and that of asthma was 9.6%, 19.0%, and 21.7% for the year 6, year 11, and year 16 surveys, respectively.

All polymorphisms studied were in Hardy-Weinberg equilibrium, with 2 exceptions (TIM1_3 and CRSP9),

both of which showed deviations that were nominally significant, with $P = .03$. Given the large number of comparisons (n = 21), these deviations were not considered statistically significant.

Table II shows the LD matrix for all polymorphisms studied. Stratifying by ethnicity did not show marked differences in the structure of the LD matrix (results not shown).

For each polymorphism, the prevalence of the genotypes in each ethnic group is presented in Table E2 (see the Online Repository in the online version of this article at www.mosby.com/jaci). Eight polymorphisms (TIMD4, TIM1_3, TIM1_4, TIM1_6, TIM3_2, ITK_2, ITK_4, ITK_5) showed significant heterogeneity by ethnicity.

The cross-sectional relations between atopy and each polymorphism at each survey are shown in Table E3 (see the Online Repository in the online version of this article at www.mosby.com/jaci). Significant associations, fairly consistent over time, were found with 1 polymorphism in TIM1 (TIM1_5), the 3 SNPs in TIM3, NM-130899, and the 2 SNPs in the 5' genomic region of ITK (see Table E3, columns 2 to 4, in the Online Repository in the online version of this article at www.mosby.com/jaci). The longitudinal analysis, taking into account information from all 3 surveys, confirmed the results of the cross-sectional analyses—that is, significant association of atopy with TIM1_5, the 3 SNPs in TIM3, the NM-130899 SNP, and the 2 SNPs in the 5' genomic region of ITK (see Table E3, column 5, in the Online Repository in the online version of this article at www.mosby.com/jaci). For most of the polymorphisms, results indicated a dominant effect, with heterozygotes and homozygotes for the rare allele showing increased risk of atopy (see Table E3 in the Online Repository in the online version of this article at www.mosby.com/jaci). Further analyses were thus performed assuming a dominant model.

Significant associations with atopy, after adjustment for multiple comparisons, were apparently clustered in 3 peaks: 1 in TIM1, 1 in TIM3, and 1 in the 5' region of ITK (Fig 1). The polymorphism in the first peak, TIM1_5, showed modest or no LD with those in the other 2 regions, whereas a moderate LD was observed between the SNPs in TIM3 and those in the 5' region of ITK (Table II). None of the associations between atopy and the polymorphisms in TIM1_5, the 3 SNPs in TIM3, and the 2 SNPs in the promoter region of ITK differed significantly according to ethnicity (data not shown).

No association was found between any of these polymorphisms and asthma at any age (Fig 2, A). The minor alleles of all 3 SNPs in TIM3 were significantly related to a higher risk for eczema, and after adjustment for multiple comparisons, 2 of these SNPs (TIM3_1 and TIM3_3) remained significantly associated with eczema, whereas TIM3_2 only reached borderline significance ($P = .06$; Fig 2, B).

To estimate the relative effects on atopy and eczema of each of the 3 SNPs in TIM3, haplotype analyses were conducted. The 3 SNPs in TIM3 were in high LD (Table II). Consequently, only 4 haplotypes out of the 8

TABLE II. Linkage disequilibrium matrix

	TIMD4	TIM1_1	TIM1_2	TIM1_3	TIM1_4	TIM1_5	TIM1_6	TIM1_7	TIM3_1	TIM3_2	TIM3_3	CRSP9	NM-130899	ITK_1	ITK_2	ITK_3	ITK_4	ITK_5	ITK_6	ITK_7	ITK_8
TIMD4		0.09	0.16	0.03	0.66	0.19	0.23	0.07	0.04	0.04	0.05	0.00	0.01	0.01	0.00	0.00	0.05	0.00	0.01	0.00	0.00
TIM1_1			0.12	0.04	0.08	0.36	0.04	0.09	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
TIM1_2				0.02	0.14	0.13	0.08	0.11	0.03	0.03	0.03	0.01	0.01	0.02	0.01	0.00	0.01	0.00	0.03	0.01	0.01
TIM1_3					0.04	0.06	0.03	0.08	0.22	0.24	0.20	0.02	0.07	0.06	0.05	0.00	0.01	0.00	0.00	0.00	0.00
TIM1_4						0.17	0.29	0.07	0.02	0.03	0.02	0.00	0.00	0.00	0.00	0.01	0.06	0.00	0.01	0.00	0.00
TIM1_5							0.10	0.00	0.12	0.15	0.15	0.02	0.07	0.07	0.06	0.00	0.01	0.00	0.01	0.01	0.00
TIM1_6								0.22	0.05	0.05	0.04	0.03	0.02	0.03	0.03	0.00	0.02	0.00	0.00	0.00	0.00
TIM1_7									0.14	0.16	0.15	0.00	0.05	0.03	0.01	0.00	0.00	0.00	0.00	0.00	0.01
TIM3_1										0.74	0.69	0.15	0.34	0.26	0.23	0.01	0.01	0.01	0.00	0.00	0.00
TIM3_2											0.87	0.19	0.40	0.37	0.29	0.00	0.02	0.00	0.00	0.00	0.00
TIM3_3												0.18	0.40	0.38	0.28	0.00	0.02	0.00	0.00	0.00	0.00
CRSP9													0.18	0.21	0.14	0.00	0.01	0.00	0.01	0.01	0.01
NM-130899														0.63	0.64	0.00	0.00	0.00	0.00	0.00	0.00
ITK_1															0.81	0.00	0.00	0.00	0.01	0.00	0.00
ITK_2																0.00	0.00	0.01	0.01	0.00	0.00
ITK_3																	0.20	0.21	0.05	0.09	0.03
ITK_4																		0.04	0.13	0.01	0.05
ITK_5																			0.26	0.02	0.01
ITK_6																				0.16	0.00
ITK_7																					0.24
ITK_8																					

$r^2 < 0.10$
 $0.10 \leq r^2 < 0.30$
 $0.30 \leq r^2 < 0.60$
 $r^2 \geq 0.60$

Each result shown is the absolute value of r^2 .

potential combinations accounted for 89.9% of all chromosomes (ATC, 80.9%; GGT, 14.4%; GTC, 2.2%; and ATT, 1.4%). Compared with the most common haplotype (ATC), the carriers of the GGT haplotype had a significant increased risk of atopy (relative risk [RR] = 1.24 [1.10-1.41]; $P = .0007$) and eczema (RR = 1.60 [1.18-2.16]; $p = 0.02$). When variation with respect to the most common haplotype occurred only in the first SNP—that is, in this case, the GTC haplotype—or only in the last SNP—that is, ATT, no significant difference in frequency of atopy and eczema was found with respect to the ATC haplotype (data not shown). The increased risk for atopy and eczema associated with the GGT haplotype was observed both in children with 2 white parents (RR = 1.27 [1.09-1.49] and 1.65 [1.10-2.47] for atopy and eczema, respectively) and in children of other ethnic backgrounds (RR = 1.19 [0.97-1.47] and 1.56 [0.99-2.46] for atopy and eczema, respectively). These results suggest that most of the association between TIM3 and allergic phenotypes is attributable to TIM3_2.

Because there was residual linkage disequilibrium between the SNPs in TIM3 described and those in the 5' region of ITK (Table II), we also assessed their relative effect on atopy and eczema. Because high LD was observed between the 2 SNPs in the 5' region of ITK ($r^2 = 0.81$), only 4 haplotypes (which accounted for 95.0% of all chromosomes) were of major interest for this purpose (Fig 3). Compared with the haplotype containing all common alleles for TIM3 and ITK, the 3 other haplotypes were associated with an increased risk for atopy, with the magnitude of the increased risk similar for all 3 haplotypes (Fig 3). However, the association did not reach statistical significance for haplotype ATCGT, most likely because of the limited frequency of that group.

These results suggest a role for SNPs in TIM3 and in ITK, independent of each other, on atopy. The association between the haplotypes and atopy did not differ by ethnic groups (P value for the interaction = .37).

In agreement with the results reported in Fig 2, B, only the haplotypes that carried rare alleles for TIM3 were related to a higher risk for eczema (RR = 1.47 [0.90-2.37] and 1.52 [1.05-2.21] for GGTAC and GGTGT, respectively). As for atopy, results did not differ by ethnic group ($P = .85$).

A haplotype analysis that also included the SNP in CRSP9, located between the TIM3 gene and the ITK gene, indicated no independent effect of this SNP on either atopy or eczema (data not shown). On the other hand, the SNP in NM-130899 was in very high LD with the 2 SNPs in the 5' region of ITK, and therefore, the separate effects of these 3 SNPs on atopy could not be determined with these data.

DISCUSSION

It is well established that genetic factors have a central role in the pathogenesis of allergic diseases.¹ Because the TIM gene family and ITK may have a role in allergy-associated immune responses^{8,9} and are located in the chromosomal region 5q33, a region repeatedly linked to asthma and allergy,³⁻⁷ we considered these genes as potential candidates for allergic phenotypes. For the first time, 3 SNPs in TIM3 were found related to atopy and eczema, and 2 SNPs in the 5' genomic region of ITK were also found to be related to atopy. We also found association between a previously reported polymorphism in TIM1 and atopy. These associations were strong enough

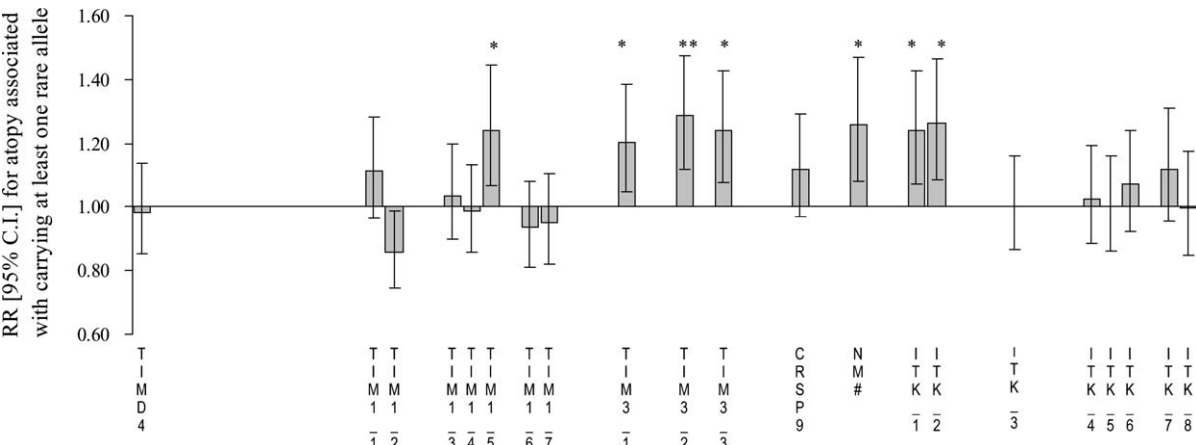


FIG 1. Relationship between atopy and each polymorphism assuming a dominant model. * $P < .05$; ** $P < .01$ (P value adjusted for multiple comparison by using the false discovery rate approach with the Benjamini and Hochberg method). #NM-130899.

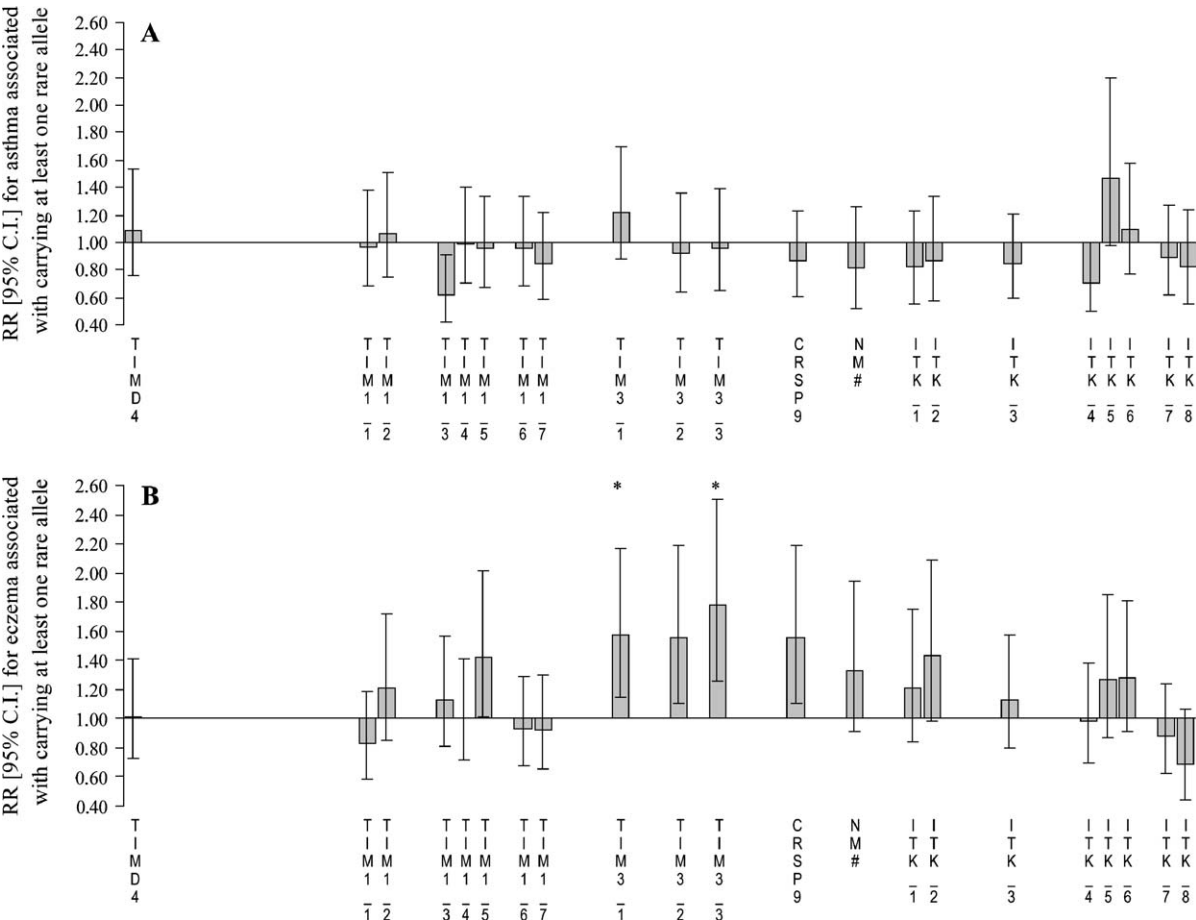


FIG 2. Relationship between asthma (A), eczema (B), and each polymorphism assuming a dominant model. * $P < .05$ (P value adjusted for multiple comparison by using the false discovery rate approach with the Benjamini and Hochberg method). #NM-130899.

to remain significant after adjustment for multiple comparisons. None of the polymorphisms studied was related to asthma. The haplotype analysis suggests independent effects of the polymorphisms in *TIM3* and in *ITK* on atopy,

although numbers of subjects were too small to reach definitive conclusions.

The analysis was performed on a large birth cohort study, a study design that minimizes selection bias. Atopy,

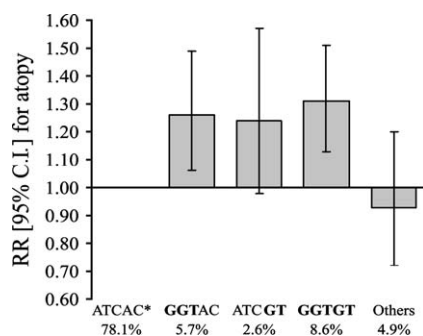


FIG 3. Relationship between atopy and haplotype in the region including TIM3_1, TIM3_2, TIM3_3, ITK_1, and ITK_2. *Reference group.

eczema, and asthma are phenotypes that vary during the lifetime, and asthma and eczema may be difficult to assess properly by questionnaire. However, the longitudinal approach conducted in this study, with phenotypic assessment at 3 different points in time during childhood, allows improvement of the phenotypic characterization, and thus of the robustness of the findings.

The TIM proteins have recently been shown to play a role in the immune responses that regulate the development of allergic diseases. TIM1 is predominantly expressed in T_H2 but not in T_H1 cells, in contrast with TIM3, which is preferentially expressed in T_H1 cells.^{10,15,27} In our study, one 15-bp insertion/deletion in exon 4 of *TIM1* (TIM1_5) was significantly related to atopy (RR for the insertion was 1.24 [1.07-1.45]) and borderline significantly with eczema (RR = 1.43 [1.01-2.01]), but not with asthma (RR = 0.95 [0.68-1.33]). However, in a Korean case-control study, Chae et al¹³ previously found an increased risk for atopic dermatitis but not for asthma with the deletion. McIntire et al¹² recently showed that the insertion was related to a decreased risk of atopy only in individuals who had past infection with HAV. However, Noguchi et al¹⁴ did not find association between polymorphisms in *TIM1* and atopic asthma. They suggested that this discrepancy may be a result of the low frequency of HAV in the young generation in Japan. Reports of the seroprevalence of HAV among school children in the United States vary, and rates are dependent on ethnic background and environmental conditions, ranging from 11% to 16.9%.^{28,29} Information regarding past HAV infection was not available in our study; thus, we could not assess whether this factor modulates the relationship observed between TIM1_5 and atopy. Further studies will be needed to elucidate this issue.

Our findings provide the first evidence of association between variants in *TIM3* and allergic diseases. Of interest, the association with atopy was particularly strong and consistent with TIM3_2 in exon 3, which predicts a putative change in the amino acid sequence of the protein (from Leu to Arg). In mice, administration of antibody against TIM3 exacerbated the severity of an experimental T_H1 -dependent autoimmune disease.¹⁵ Further experiments indicated that interaction between TIM3 and its

ligand may inhibit effector T_H1 cells and limit T_H1 -dependent immune responses.^{15,16} The 3 SNPs in *TIM3* were related to atopy and eczema, but not to asthma. This finding confirms our previous observation that different genetic factors may determine susceptibility to asthma and atopy⁷ in spite of the very high association usually observed between these 2 phenotypes in populations.

Single nucleotide polymorphisms in the 5' region of *ITK* were related to atopy in our population. The *ITK* gene encodes for an intracellular tyrosine kinase expressed in T cells. *Itk*-deficient mice have decreased T_H2 cytokine production, especially IL-4, suggesting an impaired T_H2 response.^{18,19,30} By using a murine model, Mueller and August³¹ showed that *Itk*-deficient mice have reduced lung inflammation and eosinophil infiltration after induction of allergic asthma. However, *Itk*^{-/-} mice also exhibit increased total and antigen-specific IgE.^{19,31}

Haplotype analysis was performed to attempt to distinguish the independent effects of SNPs in *TIM3* and *ITK* on atopy. Although the results suggested that the effect of the *ITK* SNPs was indeed independent from the effect of the *TIM3* SNPs, results need to be confirmed in another population because the prevalence of carriers of both common alleles in *TIM3* and rare alleles in *ITK* was small.

The major limitation of our analysis is related to the ethnic heterogeneity reflected in the Tucson population. However, similar patterns were replicated in children with 2 white parents. Thus, the significant associations observed are unlikely related to a population stratification regarding ethnicity.

Although our findings need to be replicated in other studies, our analysis strongly suggests a role for polymorphisms in *TIM1*, *TIM3*, and *ITK* in atopy and, to a lesser extent for the latter two, in eczema. Elucidating the mechanisms by which TIM1, TIM3, and ITK influence susceptibility to allergic phenotypes may help in the understanding of the pathogenesis of these complex diseases and eventually in the development of new therapeutic approaches.

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