

Genetic variants of the T-cell immunoglobulin mucin 1 but not the T-cell immunoglobulin mucin 3 gene are associated with asthma in an African American population

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Background: The T-cell immunoglobulin mucin (*TIM*) proteins and their genetic variants have been suggested to play a role in regulating allergic diseases.

Objective: Genetic association of the sequence variants for *TIM-1* and *TIM-3* genes with asthma in an African American population was investigated.

Methods: Both case-control and family-based association analyses were performed for a total of 7 polymorphisms, including 3 single nucleotide polymorphism (SNPs) and 1 insertion/deletion polymorphism in the *TIM-1* and 3 SNPs in the *TIM-3* genes. The exposure to hepatitis A virus as judged by seropositivity was also examined.

Results: In the case-control design, the frequencies of the TT genotype for SNP rs2277025 and the homozygous deletion variant (157delMTTVP) in the fourth exon of the *TIM-1* gene were higher among patients with asthma compared with the controls (odds ratio [OR], 2.779, $P = .016$; and OR, 3.09, $P = .022$, respectively). This association was substantiated by haplotype analysis of these and 2 additional SNPs (OR, 2.48; $P = .004$), and also by family-based tests for the allele and haplotype carrying 157delMTTVP ($P = .009$ and $P = .048$, respectively). Furthermore, this association seems to exist even in the hepatitis A virus–seronegative subjects in our data. None of the 3 variants in *TIM-3* genes yielded significant association with either asthma or asthma-related phenotypes.

Conclusion: Our findings suggest that the genetic variants of the *TIM-1* but not the *TIM-3* gene contribute to asthma susceptibility in this African-American population. (J Allergy Clin Immunol 2005;115:982-8.)

Key words: Asthma, single nucleotide polymorphism, T-cell immunoglobulin mucin, haplotype, hepatitis A

Abbreviations used

FBAT: Family-based association test
HAV: Hepatitis A virus
OR: Odds ratio
SNP: Single nucleotide polymorphism
Tapr: T-cell and airway phenotype regulator
TDT: Transmission disequilibrium test
TIM: T-cell immunoglobulin mucin

Genetic components are known to modulate the clinical expression of asthma as well as asthma-associated phenotypes, including bronchial hyperresponsiveness, atopy, and increased IgE concentrations.¹⁻³ Recently, the gene family of the T-cell immunoglobulin domain and mucin domain (TIM) proteins, located in chromosome 5q, has been suggested to be involved in the regulation of allergic diseases.⁴ There are 3 major family members known in human beings, *TIM-1*, *TIM-3* and *TIM-4*, which encode cell surface glycoproteins with common structural motifs, including a signal peptide, Ig domain, mucin domain, transmembrane region, and intercellular tail with phosphorylation sites. *TIM-1*, encoding the cellular receptor for hepatitis A virus (HAVCR1), is selectively expressed on activated CD4⁺ T cells and is involved in the development and regulation of T_H2-biased immune responses.⁴⁻⁶ In contrast, *TIM-3* is expressed only at the highest level on T_H1 cells and has an essential role in T_H1-mediated immune responses as well as in macrophage activation,⁷ whereas the *TIM-4* gene has not been fully characterized.⁵

Recently, McIntire et al⁴ performed a positional cloning analysis of a congenic murine model and identified variants of the *TIM-1* and *TIM-3* genes cosegregating with a T-cell and airway phenotype regulator (Tapr). On the basis of the mapping results, Tapr is shown to be genetically separable from recognized candidate genes for asthma, such as the cytokine gene cluster (IL-4, IL-5, and IL-13) and other nearby cytokine genes encoding IL-12 p40, and may be involved in the development of airway hyperresponsiveness and T_H2-mediated inflammation. Thus, human *TIM-1* and *TIM-3* were considered as possible candidate genes for asthma. A significant association has been found between 2 insertion/deletion variants in exon 4 of the *TIM-1* gene and susceptibility to atopic

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dermatitis in a Korean population,⁸ whereas the insertion variant, 157insMTTTPV, was strongly associated with protection against atopy in hepatitis A virus (HAV)–seropositive individuals.⁹ This insertion/deletion variant is located at the center of an extracellular mucin-like domain that appears to be very important in the development and the course of atopic immune response and is also required for efficient HAV uncoating.¹⁰ These results suggest, therefore, that polymorphisms in mucin domain may play an important role in modifying the activity of TIM-1.¹¹ However, Noguchi et al¹² found that none of the *TIM-1* sequence variations, including the insertion/deletion variant (157ins/delMTTTPV), were associated with atopic asthma in a Japanese population. These different results raise the possibility that the *TIM* gene family may control atopic diseases in some but not all populations, or alternatively, the HAV status of the study subjects is important in conferring susceptibility to atopic diseases.

In this report, we provide evidence that genetic variations in *TIM-1* but not in *TIM-3* genes are associated with asthma in an African American population using both population-based and family-based association analyses. Further, the variants in the *TIM-1* gene are associated with asthma even in the HAV-seronegative subjects.

METHODS

Study population

The study protocols were approved by the Institutional Review Board of Johns Hopkins University School of Medicine. Informed consent was obtained from each of the study subjects. A total of 89 African American case-parent trios were included.^{13,14} The ascertainment scheme and the clinical characteristics of these families ascertained by the Collaborative Studies on Genetics of Asthma have been described in detail elsewhere.¹⁵ Briefly, families were ascertained through an asthmatic sib-pair and asthmatic probands in case-parent trios. Each proband had to have met the following criteria: (1) at least 6 years of age; (2) either bronchial hyperresponsiveness, defined as a fall in baseline FEV₁ greater than 20% in 1 second after inhalation of less than 25 mg/mL methacholine, or reversibility, defined as a greater than 15% increase in baseline FEV₁ after inhaled bronchodilator (albuterol) for those with reduced baseline FEV₁; (3) the presence of 2 or more of the symptoms of coughing, wheezing, and shortness of breath; (4) less than 3 pack-years of cigarette smoking; and (5) a physician's diagnosis of asthma with no conflicting pulmonary disease. In the case-control design, 89 unrelated individuals were selected from among asthmatic probands of 49 multiplex African American families and 40 case-parent trios. In addition, 95 randomly selected normal healthy individuals were included (mean age, 42.8 years; range, 32–52 years; 53% male).

Single nucleotide polymorphism genotyping

Genetic analysis was conducted on either genomic DNAs or DNA amplified directly from blood by using multiple displacement amplification technology (Molecular Staging Inc, New Haven, Conn).¹⁶ Detailed information is available at <http://www.molecular-staging.com>. Single nucleotide polymorphisms (SNPs) tested for both *TIM-1* and *TIM-3* genes were selected on the basis of allele frequency and availability and retrieved from public databases at <http://www.appliedbiosystems.com>. Six common SNPs cover an 81-kb sequence, including 3 in the *TIM-1* gene spanning 25 kb and

another 3 in the *TIM-3* gene spanning 37 kb. In addition, an insertion/deletion variant, 157ins/delMTTTPV, in exon 4 of the *TIM-1* gene was also included in this study. The National Center for Biotechnology Information dbSNP, location, distance to the next SNP, and gene sequence are shown in Table I. All of these selected SNPs were genotyped in 334 African-American individuals, including 239 from 89 case-parent trios and 95 healthy volunteers. The allele frequency for each SNP is also shown in Table I. None showed evidence of departure from Hardy-Weinberg equilibrium.

Except for the insertion/deletion variant, 157ins/delMTTTPV, the primers and probes were ordered from Taqman Assays-on-Demand SNP Genotyping Products (Applied Biosystems, Foster City, Calif). Genotyping of DNA samples was performed with 5'-exonuclease (Taqman; Applied Biosystems, Foster City, Calif) assays. Taqman reactions were analyzed in a 7900HT Sequence Detection System (Applied Biosystems) with Applied Biosystems Genotyper software (SDS system, version 2.2). Each plate contained 8 duplicated samples that were randomly selected, as well as 4 no-DNA blank wells as negative controls. Accuracy of genotypes was greater than 95%, determined by concordance of DNA sequencing-derived genotypes to Taqman-derived genotypes. For 157ins/delMTTTPV, primers forward (5'-GTTTCGAACGAGCACCACCTGT) and reverse (5'-TTGTTGGAATGCTCGTTGTC) were used to amplify a 160-bp region harboring 157ins/delMTTTPV in exon 4 of the *TIM-1* gene. PCR products were purified and then applied for analysis through the ABI PRISM 3770 capillary DNA sequencer (Applied Biosystems).

Serologic assays

Serologic assays of HAV status were performed in a total of 184 participants (95 controls and 89 subjects with asthma) in the Immunology Laboratory, Department of Pathology, Johns Hopkins University School of Medicine, by using a qualitative microparticle enzyme immunoassay (IMx HAVAB; Abbott Laboratories, Abbott Park, Ill). The presence or absence of anti-HAV antibodies was defined by comparing the rate of formation of fluorescent product with the cutoff rate calculated from the analysis of the MODE 1 Calibrator provided by the supplier.

Statistical analysis

In the case-control design, genotype frequencies, odds ratios (ORs), and significance values were analyzed by using SPSS (version 11; SPSS Inc, Chicago, Ill). For these case-control data, groupwise haplotype frequencies were generated by using the Expectation Maximization algorithm.¹⁷ Haplotype frequencies and χ^2 values were calculated for each individual haplotype versus all others, and empiric significance was assessed by using 10,000 permutations of the observed data. The omnibus test was performed to detect differences in overall haplotype frequency profiles between subjects with asthma and controls.¹⁸ Total serum IgE levels between different groups were compared by means of 1-Way ANOVA. A *P* value < .05 was considered significant.

In the family-based analyses, 2 tests of association were implemented for each individual SNP: the conventional transmission disequilibrium test (TDT) as first described by Spielman et al¹⁹ and the family-based association test (FBAT) as described by Rabinowitz and Laird²⁰ with the empirical variance-covariance estimator. Haplotypes of adjacent SNPs were also used to test for evidence of linkage in the presence of association as described by Horvath et al,²¹ which assumes no recombination between markers and is analogous to the single marker statistic described by Rabinowitz and Laird,²⁰ allowing for weighting all phased genotypes (haplotypes) possible in the family. An EM algorithm that maximizes the likelihood of the phased haplotype frequencies on the basis of all of the observed family genotypes and computed under the null hypothesis was used to

TABLE I. Locations and allele frequencies of SNPs

Gene	Polymorphism	Location	Distance to next (bp)	Gene sequence	Minor allele frequency (%)
<i>TIM-1</i>	rs2277025	Intron	—	gtccagtatctcaa(c/t)ttcataatggttggtg	T (49.3)
	rs953569	Intron	2110	catacaattgttccc(g/t)ggagtitttaggcagca	T (9.7)
	157insMTTVP*	Exon4	5033	ttgtcg(ttggaacagtcgtgtca)ttggaacag	Del (36.0)
	rs1809941	Exon4	2923	tctattccagcacat(g/t)gatgtgacagctcca	T (11.7)
<i>TIM-3</i>	rs11134551	Intron	—	agagtcacacctta(a/g)gcaaaagaaattgga	G (24.0)
	novel	5'-UTR	8,816	ccaaaaccagtcag(c/g)tgacacagctcatag	G (16.9)
	rs11742259	Intron	27,881	ctcacagcatgacta(c/t)ggcgtgttcttatattg	T (36.1)

*McIntire et al.¹¹**TABLE II.** Association of *TIM-1*, *TIM-3* polymorphisms with asthma in the African American population

Gene	Polymorphism	Genotype	Asthma N (%)	Control N (%)	OR (95% CI)	P value
<i>TIM-1</i>	rs2277025	CC	19 (21.8)	36 (38.3)		
		CT	46 (52.9)	43 (45.7)	2.027 (1.013-4.058)	.045
		TT	22 (25.3)	15 (16.0)	2.779 (1.176-6.567)	.016
	rs953569	GG	70 (82.4)	75 (79.8)		
		GT	15 (17.6)	19 (20.2)	0.873 (0.474-1.607)	.662
	157insMTTVP†	Insertion	34 (37.8)	46 (49.0)		
		Insertion/deletion	40 (44.4)	41 (43.6)	1.32 (0.68-2.58)	.381
	rs1809941	Deletion	16 (17.8)	7 (7.4)	3.09 (1.05-9.82)	.022
		GG	66 (76.7)	74 (77.9)		
		GT	18 (20.9)	20 (21.6)		
<i>TIM-3</i>	rs11134551	TT	2 (2.3)	1 (1.1)	1.052 (0.614-1.802)*	.853
		AA	47 (59.5)	46 (52.9)		
		AG	28 (35.4)	39 (44.8)		
		GG	4 (5.1)	2 (2.3)	0.886 (0.654-1.201)*	.437
	novel	CC	68 (79.1)	72 (80)		
		CG	16 (18.6)	18 (20)		
		GG	2 (2.3)	—	1.029 (0.715-1.480)*	.878
	rs11742259	CC	36 (45.6)	33 (38.4)		
		CT	35 (43.3)	42 (48.8)	0.764 (0.398-1.465)	.417
		TT	8 (10.1)	11 (12.8)	0.667 (0.239-1.860)	.437

*Because of low frequency of the genotype, the group was combined with the heterozygote for this analysis.

†McIntire et al.¹¹

estimate haplotype frequencies from which these weights were determined.

RESULTS

Association analysis of *TIM-1* gene and asthma

The genotype frequency, OR, and *P* value of 3 SNPs and an insertion/deletion variant, 157ins/delMTTVP, in the *TIM-1* gene in patients with asthma and control subjects are shown in Table II. SNP rs2277025 was very common (49.3%), whereas SNP rs953569 and SNP rs1809941 showed lower frequencies (9.7% and 11.7%, respectively) in this African American population. Of interest, the insertion variant, 157insMTTVP, was reported to be rare in Koreans (13.5%), but was very common in Caucasians (58%¹¹) and in our studied African Americans (64%; Table I). Using genotype information on 184 unrelated subjects in the African American families, SNP rs2277025 was found to be in strong linkage disequilibrium with SNPs rs953569 ($D' = 1.0$) and

rs1809941 ($D' = 0.77$), as well as with the insertion variant, 157insMTTVP ($D' = 0.55$).

As shown in Table II, whereas no significant association was observed for either SNP rs953569 or SNP rs1809941, a significant difference in the genotypic frequency was found for SNP rs2277025 and the insertion variant, 157insMTTVP, between subjects with asthma and control subjects. The frequency of the heterozygous CT genotype for SNP rs2277025 was higher among subjects with asthma than controls (52.9% vs 45.7%; $P = .045$), whereas the most significant difference was seen for the homozygous TT genotype, which was 25.3% among subjects with asthma and only 16% in healthy controls ($P = .016$). Moreover, the frequency of homozygous deletion variant for 157ins/delMTTVP was significantly higher among subjects with asthma compared with controls (17.8% vs 7.4%; $P = .022$). Although analyses using haplotypes constructed across the 4 different loci yielded marginal significance for the omnibus test ($P = .073$), examination of individual haplotypes showed that the TGdelG haplotype was more frequent among

TABLE III. Association of *TIM-1*, *TIM-3*, and *TIM-1–TIM-3* haplotypes with asthma in the African American population (89 subjects with asthma, 95 controls)*

Gene	Haplotype	Asthma (%)	Control (%)	OR	P value	Omnibus LR test
<i>TIM-1</i>	C-G-ins-G	40.0	51.6	0.63	.017	0.073
	T-G-del-G	20.8	9.6	2.48	.004	
	T-G-ins-G	9.2	8.9	1.04	.923	
	C-G-del-G	7.5	8.6	0.87	.740	
	T-G-del-T	8.0	9.7	0.81	.528	
	T-T-ins-G	10.1	9.8	1.03	.925	
<i>TIM-3</i>	A-C-C	55.6	49.2	1.29	.139	0.812
	G-C-C	11.5	15.2	0.72	.421	
	G-C-T	14.2	15.7	0.89	.501	
	A-C-T	7.8	9.3	0.82	.544	
	G-G-C	7.9	6.2	1.29	.365	
	C-G-ins-G-A-C-C	28.7	34.3	0.77	.240	
<i>TIM-1–TIM-3</i>	T-G-del-G-A-C-C	11.2	0	0	.001	0.406
	T-G-ins-G-A-C-C	3.5	2.6	1.38	.760	
	C-G-ins-G-G-C-C	1.4	8.1	0.16	.040	
	C-G-ins-G-G-C-T	6.2	6.1	1.05	.928	
	C-G-del-G-A-C-C	4.4	6.6	0.66	.581	
	T-G-del-T-A-C-C	5.1	6.8	0.73	.565	
	T-G-del-G-G-C-T	5.5	2.5	2.18	.410	

*Haplotypes were analyzed for association with asthma in the African American population. The program SNPEM²⁰ was used to generate the haplotypes and their *P* values (both overall [Omnibus LR test] and specific).
LR, Likelihood ratio.

cases as compared with controls (20.8% vs 9.6%; *P* = .004; OR, 2.48; Table III). In contrast, the frequency of the CGinsG, the most common haplotype, was significantly lower among cases (40% vs 51.6%; *P* = .017; OR, 0.63; Table III).

To verify further this suggestive genetic association with risk of asthma, family-based association analysis was conducted on all 89 African American case-parent trios for each of the *TIM-1* SNPs with TDT and FBAT (Table IV). Although no evidence of linkage or association was provided for the 3 SNPs rs2277025, rs953569, and rs1809941, analysis of the deletion variant, 157delMTTTPV, demonstrated evidence of association with asthma in these African American trios (38 transmitted vs 19 nontransmitted; *P*_{TDT} = .012 and *P*_{FBAT} = .009; Table IV). Furthermore, haplotype analysis provided evidence of association with asthma for haplotype TGdelG (*P* = .048; Table IV).

Association analysis of sequence variants of the *TIM-3* gene

Among the 3 SNPs in *TIM-3*, both rs11134551 and rs11742259 are intronic, and 1 (novel) is in the 3'-untranslated region. The genotype frequencies, ORs, and *P* values of these SNPs are shown in Table II. No evidence of association was demonstrated between cases and controls in the African American population when all of these SNPs were analyzed either individually or in combination (Table III). Similar results were also observed for the family-based tests (Table IV).

TIM-1–TIM-3 haplotype analysis

Because the variants analyzed in this study are located within a region of less than 100 kb, we constructed

haplotypes across the *TIM-1* and *TIM-3* genes. Significant evidence of association with asthma was provided for a haplotype TGdelGACC, where 11.2% was observed in individuals with asthma, but none was found in healthy controls (11.2% vs 0%; *P* = .001; Table III). Further evidence was also observed for the haplotype TGdelGACC in case-parent trios when all of these haplotypes were analyzed (*P* = .036; data not shown). These results suggest that additional variants may be located within this *TIM-1–TIM-3* region or a neighboring gene in linkage disequilibrium with SNP rs2277025 and the deletion variant, 157delMTTTPV.

Effect of HAV status

Given that the insertion variant, 157 insMTTTPV, of the *TIM-1* gene has been reported to be strongly associated with protection against atopy, and that this association occurs only in individuals who are HAV-seropositive,¹¹ we performed a serological assay for HAV infection and classified all tested individuals into HAV-seronegative (*n* = 156) and HAV-seropositive (*n* = 28) groups. When the case-control sample was restricted to the seronegative subjects alone, we found that the SNP rs2277025 (data not shown) and the insertion variant, 157delMTTTPV, were marginally associated with asthma (*P* = .049; OR, 3.0; Table V). Moreover, although analyses using haplotypes constructed across the 4 and 7 different loci did not yield statistical significance for the omnibus test because of relatively limited sample size (Table V), the haplotype analyses revealed a significant association with asthma for both haplotypes TGdelG (*P* = .019) and TGdelGACC (*P* = .011). These data suggested that the association between asthma and the insertion variant 157 delMTTTPV of the *TIM-1* gene seems to exist even in the seronegative

TABLE IV. Family-based tests for association between *TIM-1* and *TIM-3* polymorphisms and asthma (89 nuclear families, 239 individuals)

TIMs	Polymorphism	Allele	Transmitted	Not transmitted	TDT <i>P</i> value	FBAT <i>P</i> value
<i>TIM-1</i>	rs2277025	T	34	33	.903	.995
	rs953569	T	25	16	.211	.615
	157insMTTVP	del	38	19	.012	.009
	rs1809941	T	10	14	.541	.396
	rs11134551	G	18	27	.233	.289
<i>TIM-3</i>	novel	G	16	13	.578	.155
	rs11742259	T	24	33	.233	.195
Haplotype		Frequency (%)		FBAT <i>P</i> value		
<i>TIM-1</i>	C-G-ins-G	37.5		.766		
	T-G-del-G	14.2		.048		
	T-G-ins-G	13.9		.183		
	T-G-del-T	11.5		.210		

TABLE V. Results of association analyses in HAV-seronegative case-control subjects

Gene	Polymorphism	Genotype	Asthma N (%)	Control N (%)	OR (95% CI)	<i>P</i> value
<i>TIM-1</i>	157insMTTVP	Insertion	32 (36.8)	30 (45.0)		
		Insertion/deletion	39 (44.8)	33 (47.8)	1.11 (0.53-2.31)	.768
		Deletion	16 (18.4)	5 (7.2)	3.00 (0.89-11.6)	.049
Gene	Haplotype	Asthma (%)	Control (%)	OR	<i>P</i> value	Omnibus LR test
<i>TIM-1</i>	C-G-ins-G	39.1	47.6	0.706	.066	0.11
	T-G-del-G	21.4	11.2	2.16	.019	
	T-G-ins-G	8.7	10.6	0.809	.580	
	C-G-del-G	7.7	8.8	0.862	.750	
	T-G-del-T	8.2	10.4	0.773	.580	
	T-T-ins-G	10.4	9.7	1.08	.833	
	C-G-ins-G-A-C-C	28.9	33.7	0.798	.363	
<i>TIM-1-TIM-3</i>	T-G-del-G-A-C-C	11.4	0	0	.011	0.427
	T-G-ins-G-A-C-C	3.6	3.5	1.02	.986	
	C-G-ins-G-G-C-C	1.5	6.6	0.211	.145	
	C-G-ins-G-G-C-T	4.9	3.9	1.29	.779	
	C-G-del-G-A-C-C	4.7	5.3	0.886	.900	
	T-G-del-T-A-C-C	5.2	8.3	0.608	.392	
	T-G-del-G-G-C-T	5.7	5.3	1.08	.923	

LR, Likelihood ratio.

subjects. We were unable to evaluate the seropositive group because of exceedingly small sample size (only 2 patients with asthma were seropositive).

Finally, we investigated the effect of these sequence variants on several asthma phenotypes for subjects with asthma whose relevant data were available ($N = 89$). No evidence of association with asthma-related phenotypes was provided for any genotypes at these loci when the total serum IgE levels, peripheral blood eosinophil counts, and methacholine PC₂₀ were analyzed by means of 1-way ANOVA, adjusted for age and sex (data not shown).

DISCUSSION

We investigated polymorphisms in both *TIM-1* and *TIM-3* genes, 2 candidate genes for asthma and allergic

diseases within a region of human chromosome 5q that has yielded linkage evidence for both atopy and asthma.⁴ Six known SNPs and an insertion/deletion variant of the *TIM-1* and *TIM-3* genes, spanning a region <100 kb, were selected on the basis of their relatively higher allele frequencies and were analyzed in an African American population using both case-control and family-based analyses. In the case-control study, 2 variants, an intronic SNP rs2277025 and an insertion/deletion polymorphism, 157ins/delMTTVP, in exon 4 of the *TIM-1* gene, showed evidence of association with asthma. This association was further substantiated when haplotype analyses were used, where haplotype (TGdelG) consisting of 4 variants in the *TIM-1* and haplotype (TGdelGACC) consisting of 7 loci (4 in *TIM-1* and 3 in *TIM-3*) were associated with asthma. Although these results suggested that haplotype analysis may have more power than the single locus test, it is also

evident that the increased significance level of association may be largely because of the T allele of SNP rs2277025 and the deletion variant, 157delMTTTPV, in the *TIM-1* gene. The observed association in case-control analysis was further supported by family-based association analyses for 157delMTTTPV, but not SNP rs2277025, when the variant was analyzed individually. Significantly, the haplotype analyses of the *TIM-1* gene or the combined *TIM-1*–*TIM-3* genes showed that the haplotype carrying the T allele of SNP rs2277025 and the deletion variant, 157delMTTTPV, was associated with asthma in case-parent trios, which can be caused by 157delMTTTPV alone or in combination with SNP rs227705. As a consequence, 157delMTTTPV seems to be more informative among these tested variants.

Although the natural ligand for *TIM-1* is currently unclear, it is known to be a receptor for HAV, and its sequence variants have been shown to be cosegregated with the *Tapr* locus in congenic mice.⁴ Subsequent to this initial discovery, several studies in human beings have been performed. McIntire et al¹¹ identified that the insertion variant, 157insMTTTPV, of the *TIM-1* gene is associated with protection against atopy, but this protection is observed only in individuals exposed to HAV. In contrast, our study demonstrated that the association of the deletion variant, 157delMTTTPV, with asthma was found even in HAV-seronegative individuals, suggesting a potential functional variation as a consequence of its interaction with an as yet undiscovered ligand. Interestingly, we observe that the association between 157delMTTTPV and asthma in our data seems to exist independent of HAV status. These results suggest, therefore, that HAV infection may confer protection against atopic diseases in subjects carrying the C allele of SNP rs2277025 and the insertion variant, 157insMTTTPV, whereas the nature of this protective effect is unclear at this time. In addition, this insertion/deletion variant is located within the mucin-like domain of TIM-1 covering O-linked glycosylation motifs,^{4,5} and it is possible that this variant modifies the glycosylation motifs and is involved in regulating the activity of *TIM-1* irrespective of HAV infection.

Of interest, Chae et al⁸ reported that the deletion variant, 157delMTTTPV, was associated with atopic dermatitis but not asthma in a case-control study in a Korean population. However, Noguchi et al¹² examined a Japanese population and demonstrated no association between *TIM-1* sequence variants, including the ins/del157MTTTPV polymorphism, and atopy using family-based analysis. In these 2 studies, the HAV status was not included in the study subjects. However, we found a significant association between the deletion variant, 157delMTTTPV, and asthma in both population-based case-control and family-based case-parent trios analyses when this variant was analyzed individually or in combination, and further, this association was found even in the HAV-seronegative subjects. These different results raise the possibility that the *TIM-1* gene may confer the risk to atopic diseases in some but not all populations, or alternatively, the HAV status of the study subjects is an

important contributing factor, as suggested by McIntire et al.¹¹ It is noted that the allelic frequency of the insertion variant, 157insMTTTPV, varies among different study populations—13.2% in Korean, 58% in Caucasian, and 64% in our African American population—which may contribute to the variable results.

In this study, the *TIM-1*–*TIM-3* haplotype showed an increased association with asthma compared with the haplotype in *TIM-1* gene only, suggesting the possible existence of additional variants in *TIM1* or neighboring genes contributing to the susceptibility to asthma. Further study is needed to identify additional variant within the region covering *TIM-1* and *TIM-3*. Also, the effect of the del/ins variants, 157ins/delMTTTPV, on *TIM-1*–mediated functional pathway needs to be investigated to establish the functional role of *TIM* sequence variants.

In summary, evidence of association with asthma for 2 variants, rs2277025 and 157ins/delMTTTPV, in the *TIM-1* gene was provided in the African American population, and this association was seen even in the HAV-seronegative subjects, suggesting that the variants in *TIM-1* are associated with asthma irrespective of the HAV infection status. Therefore, our studies of *TIM-1* variants that are likely to be of functional importance strengthen the case for a role of *TIM-1* in the pathogenesis of allergic diseases like asthma.

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