

ADAM33 polymorphisms and phenotype associations in childhood asthma

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Background: A disintegrin and metalloproteinase (ADAM) 33 has been implicated as an asthma susceptibility gene by using a positional cloning approach. However, genetic linkage of asthma phenotypes to chromosome 20p13 (the location of ADAM33) has not been observed in most asthma genome scans, and it is unclear whether these associations with ADAM33 are broadly generalizable.

Objective: To examine whether ADAM33 is associated with asthma in a North American population of childhood asthmatic patients.

Methods: We performed a family-based association study by using 652 nuclear families ascertained through asthmatic subjects enrolled in a large randomized clinical trial. Seventeen ADAM33 single nucleotide polymorphisms (SNPs; including 9 associated with asthma in the initial report) were genotyped by mass spectrometry. Single-SNP and haplotype association analysis was performed.

Results: Among white and African American subjects, no single-SNP association with asthma was observed. However, a common 16-SNP haplotype (frequency, 14.6% in white subjects) was associated with asthma ($P = .006$). Two SNPs in strong linkage disequilibrium (T1 and T+1) were marginally associated with asthma in the Hispanic cohort ($P = .04$). These data provide marginal support for an asthma locus in the ADAM33 genomic region. However, the magnitudes of the observed associations are modest at best and are inconsistent with the original report.

Conclusions: We conclude that either ADAM33 has only modest effects on asthma susceptibility, and the initial reports of association were a result of analysis in a selected population, or the initial findings were a result of chance. It is also possible

that the true asthma susceptibility locus in this genomic region is near, but not at, ADAM33. (*J Allergy Clin Immunol* 2004;113:1071-8.)

Key words: Asthma, genetics, ADAM33, metalloproteinases, association studies, haplotype

Despite the availability of a broad range of anti-inflammatory and symptom controller medications, asthma exacerbation remains the most important cause of childhood morbidity and hospitalization.¹ The identification of asthma susceptibility genes is therefore of great importance, because their elucidation will improve our understanding of the underlying pathobiology of asthma, help identify novel pathways as therapeutic targets, and provide useful diagnostic and prognostic information for individual patients. Unfortunately, positional cloning of common disease genes (including those related to asthma and allergy) remains difficult, largely because of the complex interactions of multiple genes and environmental factors.² To date, results from no fewer than 13 genome-wide linkage studies in asthma have been reported,³ but without precise localization of the disease susceptibility genes and their causative variants.

It is in this context that the publication in 2002 by Van Eerdewegh et al⁴ of their identification of a disintegrin and metalloproteinase (ADAM) 33 as an asthma susceptibility gene was received with great enthusiasm.^{5,6} A genome-wide scan using 460 white sib-pairs from the United States and the United Kingdom (UK) demonstrated highly significant linkage for asthma and airways hyperresponsiveness to a 2.5-megabase region of chromosome 20p13. By using a single nucleotide polymorphism (SNP)—based case-control association study, the investigators narrowed this region to within 185 kb, providing evidence that SNPs in ADAM33 were responsible for the association with asthma and airways hyperresponsiveness. More recently, Howard et al⁷ reported similar findings of association in 4 additional asthmatic populations, lending further support to the identification of ADAM33 as an asthma susceptibility gene. In contrast, a study of Hispanic populations found no evidence of association with asthma and 6 ADAM33 polymorphisms.⁸

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Abbreviations used

ADAM: A disintegrin and metalloproteinase
 CAMP: Childhood Asthma Management Program
 LD: Linkage disequilibrium
 SNP: Single nucleotide polymorphism
 UK: United Kingdom

We report our attempts to replicate the association of genetic variation within ADAM33 with asthma phenotypes in a heterogeneous North American population of mild to moderate childhood asthmatic subjects by using a family-based approach. Despite testing 17 polymorphisms, including 9 SNPs that were associated with asthma in the studies by both Van Eerdewegh et al⁴ and Howard et al,⁷ we were unable to demonstrate convincing evidence of single-SNP associations. Marginal evidence of association with 1 common haplotype was noted, and analysis of other asthma-related phenotypes provided some evidence that ADAM33 polymorphisms may regulate immune-related effects (including peripheral eosinophilia and total IgE levels).

METHODS

Populations

The Childhood Asthma Management Program (CAMP) is a multicenter, randomized, double-masked, placebo-controlled clinical trial designed to investigate the long-term effects of inhaled anti-inflammatory medications in children with mild to moderate asthma.^{9,10} Of the 1041 children enrolled in the original clinical trial, DNA samples were obtained from 968 participating children and 1518 of their parents. Of 652 nuclear families available for genotyping, 5 were removed from analysis because of genotype evidence of nonpaternity. Of the remaining families, 474 were of non-Hispanic white descent (436 parent-child trios, 36 sib-pairs, 2 families with 3 siblings), 66 were of African American descent (61 parent-child trios, 5 sib-pairs), and 47 were of Hispanic descent (39 parent-child trios, 8 sib-pairs). These 587 families are included in the analysis presented here. Sixty families of unspecified ethnicity were not evaluated. A diagnosis of asthma was based on methacholine hyperreactivity (PC₂₀ no greater than 12.5 mg/mL) and 1 or more of the following criteria for at least 6 months in the year before recruitment: (1) asthma symptoms at least 2 times per week, (2) at least 2 uses per week of an inhaled bronchodilator, and (3) daily asthma medication. Spirometry was performed according to American Thoracic Society recommendations by using a volume displacement spirometer, and airway responsiveness was assessed by methacholine challenge by using the Wright nebulizer tidal breathing technique.⁹ Total blood eosinophil counts were performed by using center-specific methods. Total serum IgE was measured by using radioimmunoabsorbent assays from blood samples collected during the screening sessions of the CAMP study.

Polymorphism genotyping

Single nucleotide polymorphism genotyping was performed by using unlabeled minisequencing reactions and mass spectrometry analysis (Sequenom, San Diego, Calif). Multiplex PCR and minisequencing assays were designed by using SpectroDESIGNER software (Sequenom). 3-5 plex PCR reactions were performed by

using 2.5-ng genomic DNA in 5 μ L (protocol details and primer data are available at <http://wchanning.bwh.harvard.edu/epigenetics/Projects>). Secondary multiplex single-primer minisequencing reactions were performed and analyzed by using the Bruker Bi-flex MALDI-TOF mass spectrometer (Bruker Daltonics, Billerica, Mass). Spectral output was analyzed by using SpectroTYPER-RT software (Sequenom) and by manual review.

Genotype data quality control

Duplicate genotyping was performed on approximately 10% of the cohort to assess genotype reproducibility. Genotype data quality was assessed by using several criteria, including completeness of genotype data, degree of discordant duplicate genotyping, evidence of excessive non-Mendelian marker inheritance (by using FBAT^{11,12}), and Hardy-Weinberg disequilibrium among parental data by using an exact method.¹³ On the basis of this evaluation, assays for 3 loci (KL+1, V2, and ST+7 from Van Eerdewegh et al⁴) produced unreliable genotype data (high number of Mendelian inconsistencies and high discordance rates) and were not considered for further analysis.

Statistical analysis

Pairwise linkage disequilibrium between each pair of SNP loci was evaluated by using a maximum likelihood method¹⁴ to infer phase for dual heterozygotes and was expressed as r^2 .¹⁵ Haplotypes were inferred by using Bayesian methods¹⁶ as implemented in the Phase package.¹⁷ Haplotype block structure was determined by using HaploBlockFinder (<http://cgi.uc.edu/cgi-bin/kzhang/haploBlockFinder.cgi>).¹⁸

Tests of association with asthma were evaluated by using FBAT version 1.4. Quantitative trait analysis was performed by using PBAT^{19,20} for 4 intermediate phenotypes: percent predicted post-bronchodilator FEV₁, airway hyperresponsiveness to methacholine (log-transformed PC₂₀), total serum IgE levels (log-transformed), and total blood eosinophils (log-transformed). Evidence for haplotype association with asthma was assessed by using the likelihood ratio score test implemented in TRANSMIT.²¹ Tests for global significance of all haplotypes were performed. Haplotype analysis was restricted to the white cohort given the limited sample size for the other ethnic groups. Power calculations were performed by using PBAT assuming a population disease prevalence of 10% and a genetic attributable fraction for the disease allele of 0.1, and assuming that markers tested were disease susceptibility loci.

SAS version 8.2 (SAS Institute, Cary, NC) and Web-based bioinformatic tools (<http://innateimmunity.net>) were used to manage and analyze the data. Statistical significance was defined at the 5% level.

Human subjects

Informed assent and consent were obtained from the study participants and their parents to collect DNA for genetic studies. The Institutional Review Board of the Brigham and Women's Hospital, as well as those of the other CAMP study centers, approved the study.

RESULTS

In our attempt to replicate evidence of association with ADAM33 polymorphisms and asthma, we genotyped 20 ADAM33 SNPs in 587 nuclear families ascertained through an asthmatic proband. The phenotypic characteristics of the 640 asthmatic children are presented in Table I. Three of the SNP genotyping assays (KL+1, V2, and ST+7 from Van Eerdewegh et al⁴) did not produce reliable genotype data and were excluded from further

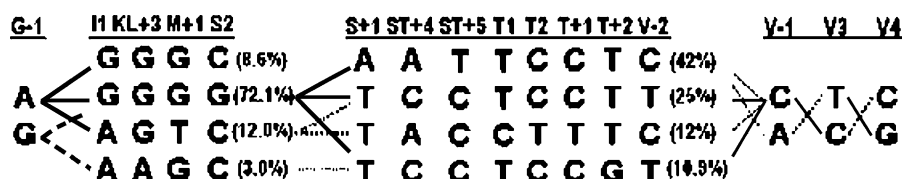


FIG 1. Haplotype block structure of ADAM33. Determination of haplotype block structure was performed using HaploblockFinder.¹⁸ Blocks were defined by Minimal ID'I. Lines denote transitions between blocks and connect blocks that were observed in combination in the population. Percentages in parentheses denote the estimated frequency of each block-specific haplotype.

TABLE I. Phenotype characteristics of CAMP asthmatic children included in analysis

Variable	CAMP asthmatic subjects (n = 640)*
Sex	
Male	403 (63.0%)
Female	237 (37.0%)
Race	
White	514 (80.3%)
African American	71 (11.1%)
Hispanic	55 (8.6%)
Mean age, y	8.15 (2.11)
Median age of onset of asthma symptoms, y	3.5 (2.0-5.0)
Mean FEV ₁ , percent predicted	102.9 (12.6)
Median PC ₂₀ , mg/mL	1.01 (0.73-1.51)
Median total serum IgE, IU/mL	426.6 (177-1174.9)
Median total blood eosinophils, cells/mm ³	407.4 (209-676)

*Values in parentheses indicate percentage, SD, or midquartile range, where applicable.

analysis. All of the remaining 17 SNPs were in Hardy-Weinberg equilibrium among the parents, with the exception of SNP V-2 among African American parents, which was marginally out of Hardy-Weinberg equilibrium because of an excess of heterozygotes (62 observed compared with 51 expected; exact *P* value = .04).

Allele frequencies, linkage disequilibrium, and haplotype structure of ADAM33

The minor allele frequencies observed among the probands for the 17 ADAM33 loci evaluated are presented in Table II. Fifteen of 17 SNPs genotyped were also assessed by Van Eerdewegh et al,⁴ including 9 that demonstrated significant associations with asthma in their study. Two additional polymorphisms were genotyped: rs615436 (a nonsynonymous SNP coding for a substitution of tyrosine for arginine at amino acid 515, here referred to as *N1*, following the naming convention used by Van Eerdewegh et al⁴), and rs2280093 (an intronic SNP located 309 bases downstream from exon 11, here referred to as *KL+3*). The *N1* polymorphism was observed only in the African American population, whereas the *KL+3* polymorphism was observed primarily among white and Hispanic subjects. The allele frequency

distributions were similar between the CAMP white and Hispanic subjects and differed substantially from those in the African American probands. Similar findings were observed when examining linkage disequilibrium (LD) patterns. We compared the allele frequency distributions between the CAMP white probands and those reported by Van Eerdewegh et al.⁴ In general, the allele frequencies observed in the CAMP white probands more closely resembled those of the white UK populations than the white US populations, and more often resembled the allele frequencies observed among the controls (Table II).

Ethnicity-specific pairwise LD was assessed for all loci by using parental genotype data. LD was similar between white and Hispanic subjects, but specific pairwise differences were noted in the African American population. In general, LD across ADAM 33 was not strong and did not extend across the gene regardless of population origin, although typically, LD was even less in the African American population between all loci (see figure of LD plots at <http://wchanning.bwh.harvard.edu/epigenetics/Projects>). ADAM33 haplotypes imputed from parental data with a frequency of 1% or more are presented in Table III. A total of 19 haplotypes were observed, of which 8 were common to all 3 ethnic groups. These 8 haplotypes represent 85% of all white haplotypes identified. Haplotype diversity was greater among Hispanic subjects (12 common haplotypes, 83% of total) and even greater among African Americans (17 haplotypes, 68% of total). The block structure of white haplotypes is presented in Fig 1. Two discrete blocks were observed: one 4-SNP block (block 1) and an adjacent 8-SNP block (block 2). Within each block, only 4 haplotypes were observed with frequencies of >1%, suggesting minimal within-block recombination. Conversely, there was evidence of substantial recombination between the 2 blocks, and SNPs that flanked the 2 blocks (G-1, V-2, V3, and V4) were largely dissociated, suggesting the presence of recombination hot spots within ADAM33.

Association studies

Tests of association with asthma and each of the 17 SNPs were performed by using the FBAT family-based test of association (Table IV). We were unable to detect significant evidence of association to asthma with any of the SNPs tested among the white sample. Similarly, no significant association was observed in the African American cohort. Among the Hispanic subjects, 2 loci in

TABLE II. ADAM33 polymorphisms genotyped in CAMP*

dbSNP rs#	SNP name	Location	Alleles	Minor allele frequency						
				CAMP probands			Van Eerdewegh et al4			
				White (n = 474)	African American (n = 66)	Hispanic (n = 47)	US		UK	
							Cases	Controls	Cases	Controls
rs2485700	G-1	IVS6 -62	A>G	0.147	0.284	0.132	0.038	0.082	0.099	0.098
rs2271511	I1	Gly288Gly	G>A	0.18	0.403	0.208	0.296†	0.128†	0.108†	0.163†
rs2280093	KL+3	IVS11 +309	G>A	0.032	0.007	0.028	—	—	—	—
rs3918395	M+1	IVS13 +35	G>T	0.137	0.127	0.113	0.222†	0.080†	0.082†	0.130†
rs615436	N1	Arg515Tyr	A>G	0	0.096	0	—	—	—	—
rs528557	S2	Gly717Gly	G>C	0.253	0.669	0.302	0.352†	0.247†	0.160†	0.271†
rs2853209	S+1	IVS19 +181	T>A	0.454	0.25	0.412	0.609	0.481	0.505	0.531
rs44707	ST+4	IVS19 +427	A>C	0.425	0.394	0.453	0.365†	0.429†	0.408†	0.519†
rs597980	ST+5	IVS19 +488	C>T	0.44	0.209	0.385	0.444	0.500	0.500	0.444
rs2280091	T1	Met764Thr	T>C	0.14	0.172	0.125	0.241†	0.078†	0.087†	0.132†
rs2280090	T2	Pro774Ser	C>T	0.138	0.148	0.12	0.204†	0.074†	0.082†	0.105†
rs2280089	T+1	IVS20 +66	C>T	0.129	0.138	0.135	0.200†	0.080†	0.089†	0.131†
rs630712	T+2	IVS20 +127	T>G	0.118	0.069	0.235	0.056	0.104	0.125	0.125
rs628965	V-2	IVS21 -66	C>T	0.397	0.338	0.4	0.333	0.353	0.393	0.376
rs543749	V-1	IVS21 -32	C>A	0.102	0.235	0.12	0.130†	0.171†	0.062†	0.136†
rs677044	V3	3UTR: c.2620	T>C	0.21	0.235	0.219	0.278	0.234	0.199	0.215
rs2787094	V4	3UTR: c.2891	C>G	0.184	0.311	0.222	0.167†	0.208†	0.163†	0.246†

*SNP names refer to those used by Van Eerdewegh et al.⁴ SNP location with reference to mRNA GenBank RefSeq NM_025220. Minor allele frequencies refer to those of the second allele listed. Allele frequencies from Van Eerdewegh et al⁴ abstracted from online supplement that accompanied report (<http://www.nature.com/nature>).

†Frequencies significantly different between cases and controls in initial report.

strong LD with one another (T1 and T+1) demonstrated marginal evidence of transmission distortion (P values .03 and .02, respectively), despite the very small number of informative Hispanic families available (17 and 15, respectively). These results are not significant when corrected for multiple comparisons. Moreover, among the white and African American families, the allelic transmissions trended in the opposite direction (the minor allele was undertransmitted), suggesting that the associations observed in Hispanics are either ethnicity-specific or simply a result of chance. To assess whether ADAM33 polymorphisms were related to intermediate asthma-associated phenotypes, quantitative trait analysis was performed by using PBAT with an additive model for 4 quantitative traits: postbronchodilator FEV₁ (percent predicted), log-transformed PC₂₀, log-transformed blood eosinophil levels, and log-transformed total serum IgE levels. SNP T+2 demonstrated evidence of association with blood eosinophil levels among white subjects ($P = .03$). This locus was estimated to contribute 2.3% of the total heritability of blood eosinophilia. T1 and T+1 were associated with serum IgE and eosinophil levels among Hispanics subjects (unadjusted P values, .04; data not shown). Importantly, no evidence for association with airway responsiveness or FEV₁ was observed in any ethnic group.

In an attempt to recapitulate the haplotypic association analysis performed by Van Eerdewegh et al.,⁴ 3 types of analyses were performed by using the white trios: analysis of the complete 16-SNP haplotype, pairwise haplotype analysis, and haplotype block analysis. Overall, there was

little evidence of association. The global tests of haplotype association did not reach statistical significance for the 16-SNP analyses, the pairwise comparisons, or the block studies. These results suggest that the ADAM33 haplotypes are not strongly associated with asthma. However, global tests of haplotype association are conservative in that subtle effects of individual haplotypes may not be detected. To assess for weaker haplotype-specific effects, we repeated the 16-SNP analysis by using a biallelic test, in which individual haplotypes were assessed for transmission-distortion. By using this strategy, 1 common haplotype (haplotype 2 in Table III) was overtransmitted to affected offspring ($P = .006$). Importantly, these effects were not observed when either pairwise haplotypes or individual haplotypes within blocks were assessed. Given the lack of significance of the global test and the large number of haplotype comparisons performed, and the observation that strong LD does not extend across ADAM33, the true importance of this extended haplotype association is questionable.

DISCUSSION

ADAM33 is an attractive candidate as an asthma susceptibility gene. The gene belongs to a large family of molecules (disintegrin-containing zinc-dependent metalloproteinases) implicated in diverse biological processes, including cell fusion, myofibroblast proliferation, proteolysis, and cell signaling.⁶ ADAM33 is primarily expressed in smooth muscle, and within lung tissue

TABLE III. ADAM33 haplotype structure in CAMP*

	SNP loci																Haplotype frequency			
	G-1	I1	KL+3	M+1	N1	S2	S+1	ST+4	ST+5	T1	T2	T+1	T+2	V-2	V-1	V3	V4	White (n = 994)	Hispanic (n = 92)	African
																				American (n = 129)
(1)	A	G	G	G	A	G	A	A	T	T	C	C	T	C	C	T	C	33	33	4
(2)	•	•	•	•	•	•	T	C	C	•	•	•	•	T	•	C	•	15	8	4
(3)	•	A	•	T	•	C	T	•	C	C	T	T	•	•	•	•	•	12	7	8
(4)	•	•	•	•	•	•	T	C	C	•	•	•	G	T	•	•	G	10	6	3
(5)	•	A	•	•	•	C	T	•	C	•	•	•	•	•	•	•	•	—	1	15
(6)	•	•	•	•	•	•	T	C	C	•	•	•	G	T	•	•	•	—	13	1
(7)	G	•	•	•	•	•	T	C	C	•	•	•	•	T	•	•	•	7	3	1
(8)	G	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	1	2	8
(9)	•	•	•	•	•	C	•	•	•	•	•	•	•	•	A	•	G	6	2	2
(10)	•	•	•	•	G	C	•	•	•	•	•	•	•	•	A	•	•	—	—	8
(11)	G	A	•	•	•	C	T	C	C	•	•	•	•	T	•	C	•	1	1	1
(12)	G	•	•	•	•	•	T	C	C	•	•	•	•	T	•	C	•	—	—	3
(13)	G	A	A	•	•	C	T	C	C	•	•	•	•	•	A	C	•	—	2	—
(14)	•	•	•	•	•	•	T	C	C	•	•	•	G	•	A	C	G	—	2	—
(15)	•	•	•	•	•	C	T	C	C	•	•	•	•	•	A	C	•	—	—	2
(16)	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A	•	G	—	—	2
(17)	•	•	•	•	•	•	•	•	•	•	•	•	•	•	A	•	•	—	—	2
(18)	G	A	•	T	•	C	•	•	•	C	T	T	•	•	•	•	•	—	—	2
(19)	G	•	•	•	•	•	T	C	•	•	•	•	G	T	•	•	G	—	—	2
Percent of total																		85	80	68

*Parental haplotypes observed at least twice in CAMP cohort are presented. Haplotypes imputed using Phase.17 *Dot* denotes common allele.

localizes to both vascular and bronchial smooth muscle.^{4,22} Moreover, the murine homologue, Adam33, maps to mouse chromosome 2, a region that harbors an innate airways responsiveness quantitative trait locus.²³ Given the initial findings of association and linkage among asthmatic subjects with airways hyperresponsiveness, it has been speculated that ADAM33 primarily influences airways hyperresponsiveness.²⁴ However, because other ADAM proteins (ADAM10 and ADAM17) appear to interact with inflammatory cytokines (including TNF- α), it has been speculated that ADAM33 may also have important cytokine-stimulating effects.²⁵ However, currently, the only evidence of ADAM33 as an important molecule in asthma pathogenesis is the linkage and association data. Given our weak evidence of association between ADAM33 polymorphism and asthma, it is worthwhile to review the previous evidence in support of ADAM33.

The initial report by Van Eerdewegh et al⁴ provided convincing evidence that a 2.5-megabase region on chromosome 20q13 is linked to asthma and airways responsiveness. The evidence for linkage was strong, meeting the stringent criteria of genome-wide significance.²⁶ Additional fine-mapping SNP association analysis provided further evidence for ADAM33: SNPs within the gene demonstrated statistically strong association (*P* values ranging from .04 to .000003, uncorrected for multiple comparisons) in case-control and family-based studies using the US and UK families that provided the evidence of linkage on chromosome 20p. Despite these rather impressive results, several important internal inconsistencies should be noted. First, no single-SNP

was associated with asthma in both the UK and US cohort. In the UK cohort, SNPs F+1, Q-1, S1, S2, ST+4, V-1, and V4 were significantly associated with asthma, whereas SNPs I1, L-1, M+1, T1, T2, and T+1 were significant in the US cohort. Although allelic heterogeneity is a possible explanation for these results, this pattern of inconsistency should be observed only for very rare variants that are not present (or are present at very low frequency) in 1 of the populations. All variants assessed were seen in both the UK and US populations, at relatively common frequencies. If these are truly susceptibility alleles, many (particularly the most common alleles) should demonstrate association in both populations. A second concern is that only 1 (S1) of 12 SNPs that demonstrated significant association in the combined case-control study also demonstrated significant transmission distortion in the family-based analysis. None of the other 12 SNPs associated in the case-control studies were replicated in the family-based analysis, suggesting that the results may be caused in part by population substructure—a potentially important cause of false-positive results in case-control genetic studies.^{27,28} Finally, although the family-based analysis suggested impressive haplotype associations, the vast majority of these haplotypes were not associated with asthma in the case-control analyses. Moreover, additional analyses presented with restriction of cases to either those with airways responsiveness alone or a composite of airways responsiveness and asthma were unable to replicate any haplotype associations in the case-control design. It is therefore unclear which variants or combinations of variants in ADAM33 actually contribute to the linkage signal initially presented. Replication of

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consistent allelic associations in additional populations would therefore help to resolve this issue.

By using a case-control study design, Howard et al⁷ reported evidence of association replication of ADAM33 and asthma in 4 distinct cohorts: a US white cohort, a Dutch white cohort, an African American cohort, and a Hispanic cohort. Eight SNPs were tested (S1, S2, ST+4, ST+7, T1, T2, V-1, and V4). Several SNPs showed evidence of association in each of the cohorts with asthma, and with atopy-related phenotypes, including skin test reactivity and serum IgE. Importantly, 3 SNPs demonstrated evidence of association in more than 1 population. Among the 2 white populations, SNP ST+7 (rs574174) was associated with asthma ($P = .009$ in Dutch; $P = .017$ in US). This SNP was also significantly associated with asthma in the original report by Van Eerdewegh et al⁴ when the UK and US populations were combined ($P = .02$), but not in each population separately ($P = .05$ in UK; $P = .31$ in US). ST+7 is an intronic SNP located 410 bases upstream from the nearest exon donor site and has no clear functional consequence. ST+7 was unfortunately not genotyped in the Hispanic cohorts evaluated by Lind et al.⁸ The polymorphism was genotyped in our cohort but did not produce reliable results on quality control and was therefore not analyzed for evidence of association. However, on the basis of the haplotype distributions described in Howard et al,⁷ ST+7 appears to be in very strong LD with V-1 in both white and Hispanic subjects. V-1 was genotyped in both the Hispanic cohort of Lind et al⁸ and our cohort, and was not associated with asthma or associated phenotypes in either population.

Lind et al⁸ recently reported the results of their evaluation of 8 ADAM33 SNPs in 583 Mexican asthmatic trios, 190 Mexican and 183 Puerto Rican asthmatic subjects, and 325 ethnically matched controls.⁸ They were unable to demonstrate associations with asthma or asthma severity phenotypes in the Mexican populations. In the Puerto Rican sample, SNPs V-1, V1, and V4 demonstrated marginal associations with measures of asthma severity (uncorrected P value, .039). These SNPs were not associated with asthma diagnosis in this cohort and notably were not related to asthma in the study by Howard et al.⁷ One SNP that did demonstrate associations in the Hispanic cohort of Howard et al⁷ (SNP T2; P value = .04) did not replicate in the Hispanic populations of Lind et al.⁸

We were not able to replicate ADAM33 single-SNP associations with either asthma or airways responsiveness in a large population of childhood asthmatic subjects representative of a North American population with mild to moderate asthma. We tested 17 polymorphisms, 9 of which have been associated with asthma in the original report. The only statistically significant results were weak associations between T+2 and total eosinophil count ($P = .02$) in white subjects and T1 and T+1 with asthma ($P = .03$ and .02, respectively) and IgE and eosinophilia (both P values = .04) in Hispanic subjects. These results are not significant when corrected for multiple testing. In addition, these associations were not observed in the

TABLE IV. Family-based associations for ADAM33 SNPs*

SNP	Allele	Transmission:untransmission ratio			
		White trios	African American trios	Hispanic trios	Overall
G-1	G	117:120	19:26	8:17	144:163
I1	A	122:140	24:24	18:11	164:175
KL+3	A	27:33	NI	3:5	30:38
M+1	T	113:113	11:10	10:3	134:126
N1	G	NI	10:9	NI	10:9
S2	C	169:149	24:29	11:12	204:190
S+1	A	204:213	23:20	17:17	244:250
ST+4	C	216:203	36:30	15:24	267:257
ST+5	T	204:212	17:17	18:20	239:249
T1	C	106:122	12:14	13:4†	131:140
T2	T	104:111	16:17	11:4	131:132
T+1	T	79:97	10:13	12:3†	101:113
T+2	G	86:79	8:6	10:15	104:100
V-2	T	190:163	30:25	14:15	234:203
V-1	A	62:70	19:20	3:10	84:100
V3	C	110:100	16:25	7:11	133:136
V4	G	120:126	22:29	10:11	152:166

NI, Noninformative.

*Raw counts of transmitted and untransmitted alleles among informative families.

†Significant at $P < .05$.

Hispanic cohorts evaluated by Howard et al⁷ and Lind et al.⁸ Detailed haplotype analysis was generally negative as well. No pairwise haplotypes were significantly associated with asthma, and haplotype block association studies were also negative. When all 16 SNPs present in white subjects were considered simultaneously, haplotype 2 demonstrated transmission distortion (biallelic uncorrected P value, .006), although the more conservative global significance test was not significant. At best, these results suggest that haplotype 2 is in LD with an untyped disease susceptibility SNP. If so, it is unlikely to be ST+7, given that the G allele (overrepresented among cases in the studies by both Van Eerdewegh et al⁴ and Howard et al⁷) is not unique to haplotype 2 (inferred from the haplotype data of Howard et al⁷) but segregates on all of the common haplotypes.

On the basis of the results from these 4 studies, what can we conclude regarding the role of ADAM33 polymorphisms in asthma? Failure to replicate genetic associations in complex disease is, unfortunately, a common occurrence. Meta-analyses of association studies suggest that the most frequent causes for replication failure include false-negative association because of statistically underpowered replication studies, positive publication bias of associations with marginally significant results that may be caused by chance, phenotypic and genetic heterogeneity, and overestimation of true effect size in initial reports (the so-called *winner's curse effect*).^{29,30} The cohorts of Lind et al⁸ and the CAMP cohort were larger than those that demonstrated allelic associations and were sufficiently powered to identify allelic effects of magnitude similar to those initially reported, particularly given the

relatively high minor allele frequencies of the associated polymorphisms (0.10-0.425). In CAMP, power was at least 0.85 to detect an association with asthma with the SNPs with minor allele frequencies of 0.1 or greater (such as $V=1$), presuming this locus was responsible for 10% of the genetic attributable risk for asthma. CAMP is not sufficiently powered to detect associations of substantially weaker effect. Despite high statistical significance of the associations presented by Van Eerdewegh et al,⁴ the possibility that their findings were a result of chance cannot be excluded, given the very large number of allelic and phenotypic comparisons performed as well as the internal inconsistency of the allelic associations in the US and UK populations. Because the majority of the associations observed were with common polymorphisms (frequencies >5%) and the allele frequencies and haplotype distributions were very similar between the cohorts, genetic heterogeneity is an unlikely explanation for the discrepant results. However, genetic heterogeneity may play an important role if the true pathogenic variants are located at some distance from the ADAM33 locus and the associations observed are caused solely by LD. In that case, longer-range LD patterns may be very different in these cohorts and would affect tests of association with ADAM33 SNPs. We did not genotype SNPs at a distance from ADAM33 and are unable to exclude this possibility.

Phenotypic and environmental heterogeneity between cohorts is an important cause of inconsistent association and should not be overlooked as an explanation for the results. However, for the most part, the asthma definitions and proband characteristics across studies are quite similar. Except for the Dutch cohort in the study by Howard et al,⁷ all studies focus on childhood asthma. The ascertainment schema for the CAMP study, the study by Van Eerdewegh et al,⁴ and the study by Lind et al⁸ were very similar. All 3 required a physician diagnosis of asthma and active use of asthma medication. Although methacholine responsiveness was not a requirement for entry into the study by Van Eerdewegh et al⁴ (but was for CAMP), peak linkage on chromosome 20p in the study by Van Eerdewegh et al⁴ was maximized when the sample was restricted to those subjects with airways hyperresponsiveness ($PC_{20} \leq 16$ mg/mL). Similarly, the asthmatic subjects studied by Howard et al⁷ (white, African American, and Hispanic subjects) all demonstrated airways hyperresponsiveness and reported 2 or more asthma symptoms. Importantly, features of atopy (high total IgE, specific IgE, or allergen-induced skin test positivity) were common across all cohorts. Therefore, although it is well known that asthma is a clinically heterogeneous disorder, there is evidence that the cohorts discussed here are fairly similar. It is important to note that the linkage peak on chromosome 20p13 has not been identified as a major locus in any of the other 13 genome-wide linkage scans in asthma and was found to demonstrate marginal evidence ($P = .04$) for linkage in only 1 study.³¹ Although ADAM33 polymorphisms may contribute to asthma pathobiology, the lack of frequent linkage across studies suggests that in most populations,

the gene effects are small, and they may be important only in populations with unique (unidentified) characteristics.

In summary, ADAM33 polymorphisms were not associated with asthma or related phenotypes in a large North American population of childhood asthmatic subjects. Although in keeping with similar results among Hispanic subjects, these results are in sharp contrast with those of 2 groups that have demonstrated associations, and raise doubt regarding ADAM33 in asthma pathogenesis. It is important to note that functional effects with ADAM33 variants have not been described. It is unclear whether the asthma-associated variants have any effect on ADAM33 expression, structure, or function. We propose that in addition to testing ADAM33 SNPs in more populations, functional analysis of these polymorphisms is warranted and should specifically address molecular mechanisms that may confer asthma susceptibility. Also, further testing of SNPs in genes surrounding ADAM33 is warranted.

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