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A Six-SNP Haplotype of *ADAM33* Is Associated with Asthma in a Population of Cartagena, Colombia

Candelaria I. Vergara[a, b, d] Nathalie Acevedo[a, b] Silvia Jiménez[a, b] Beatriz Martínez[a] Dilia Mercado[a] Leonor Gusmão[c] Kathleen C. Barnes[d] Luis Caraballo[a, b]

[a]Institute for Immunological Research, University of Cartagena, and [b]Fundemeb Foundation for the Development of Medical and Biological Sciences, Cartagena, Colombia; [c]Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Porto, Portugal; [d]Division of Allergy and Clinical Immunology, Department of Medicine, Johns Hopkins University, Baltimore, Md., USA

Key Words
A disintegrin and metalloprotein 33 · *ADAM33* · Immunoglobulin E · Asthma · Allergy · Colombians

Abstract
Background: A disintegrin and metalloprotein-33 *(ADAM33)* participates in the bronchial remodeling process in asthma, and genetic analyses pointed it out as a candidate gene in asthma. ***Methods:*** To analyze the association between *ADAM33* and asthma and total and mite-specific IgE levels in a population of the Caribbean Coast of Colombia, we genotyped 6 single-nucleotide polymorphisms of *ADAM33* in 429 asthmatics, 401 controls and 116 family trios using fluorogenic probes. Total and specific IgE against *Blomia tropicalis* and *Dermatophagoides pteronyssinus* were determined by ELISA. Case-control and family-based analyses were performed. Case-control association analyses were corrected by population stratification using a set of 52 ancestry-informative markers. ***Results:*** Eight common haplotypes were identified; among them, H4 (GCAGGG) was associated with asthma in the family group (Z score: –2.049, p = 0.04). We also found an association between the TT genotype of ST+7 and asthma in the case-control study (p = 0.05) that disappeared after correcting for multiple testing. In the family-based analysis, this genotype was a risk factor for asthma (p = 0.01), high total IgE (Z score: 2.546, p = 0.01) and high specific IgE against *B. tropicalis* (p = 0.02) and *D. pteronyssinus* (Z score: 2.414, p = 0.01). V4 was associated with specific IgE against *B. tropicalis* (p = 0.03); T2 with asthma (p = 0.03), high total IgE (p = 0.02) and IgE against D. *pteronyssinus* (p = 0.03) and T1 with high total IgE (p = 0.04). None of these associations was maintained after correction for multiple testing. ***Conclusions:*** Our findings suggest a relevant role of *ADAM33* in the pathogenesis of asthma in this population.

Introduction

Asthma is an atopic disease characterized by chronic lung inflammation, bronchial hyperresponsiveness (BHR) and airway remodeling [1, 2]. The disorder involves complex interactions between genetic and environmental factors. Although the allergic response has been considered as a major risk factor for asthma onset

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Correspondence to: Prof. Luis Caraballo
Institute for Immunological Research
University of Cartagena
Cra. 5 # 7–77, Cartagena (Colombia)
Tel. +575 665 1876, Fax +575 669 8491, E-Mail luiscaraballo@telecom.com.co

and development, airway remodeling, defined as changes in the airway structure that increase the predisposition to allergic sensitization, is also an important component in the process leading to asthma.

A disintegrin and metalloprotein-33 *(ADAM33)* was one of the first asthma candidate genes identified by positional cloning [3]. The original investigation describing it as a risk factor for asthma in a Caucasian population has been followed by a large number of replications with diverse and dissimilar results, as is often the case in this kind of studies. Given its similarity with other molecules of the *ADAM* family, it has been proposed that *ADAM33* plays an important biological role as an activator of growth factors and Th2 cytokines [4]. Additionally, some recent analyses showed the expression and epigenetic regulation of this gene in bronchial epithelia and smooth muscle cells of the lung [5, 6]; moreover, higher expression has been described in asthmatic bronchial tissue compared to nonasthmatic tissue [3]. Therefore, although the genetic analysis has been inconclusive individually, taking into account all the existing evidence, *ADAM33* turns out to be one of the most probable candidates as a susceptibility gene for asthma and other diseases with a strong immunological component.

One of the fundamentals in genetic epidemiologic research is replicating new findings of association studies in different populations. As *ADAM33,* other candidate genes and their polymorphisms have been associated with asthma in case-control and family-based studies; however, only a few of these associations have been successfully reproduced in follow-up studies [7, 8]. Although this gene has been analyzed in Latin-American populations, its association with asthma or allergy has not been investigated in any South American population. We aimed to elucidate its role in asthma and to assess total (tIgE) and specific (sIgE) IgE levels in a population of Cartagena (Colombia) which has a high prevalence of the disease [2, 9], lives in a tropical environment and is remarkably exposed to mite allergens [10, 11].

Methods

Subjects

Two independent datasets were used. The first comprised 429 nonrelated adult asthmatics and 401 controls (mean age 36.15 ± 18.32 and 34.98 ± 17.8 years, respectively) and the second included 116 family trios consisting of 2 parents and 1 asthmatic proband (348 individuals). The subjects were recruited from the Social Security Clinic and outpatient health centers of Cartagena, a tropical city of the Caribbean Coast of Colombia. A full verbal explanation of the investigation was provided and all subjects gave written consent as previously approved by the Bioethics Committee of the University of Cartagena. Asthma was defined according to the Global Initiative for Asthma criteria [12] using a standardized questionnaire previously tested [2, 9] in patients with a history of physician-diagnosed asthma as previously described [13] and including a small number of subjects (n = 105) with pulmonary function tests. A physician belonging to the research staff confirmed the diagnosis. Subjects meeting the following criteria were recruited: current asthma, ≥8 years old and a history of ≥2 years of asthma, ≥3 episodes of asthma symptoms (wheezing, chest tightness and dyspnea) in the last 12 months or absence of symptoms due to the use of antiasthmatic medications. Controls were individuals with no family or personal history of asthma and allergies. The family trios were ascertained through the asthmatic offspring who met the mentioned criteria. All participants resided in Cartagena, Colombia.

IgE Measurements

tIgE levels were measured in all individuals using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Ridascreen® Total IgE; R-Biopharm, Darmstadt, Germany). Since *Blomia tropicalis* and *Dermatophagoides pteronyssinus* are the main source of sensitization in tropical environments [14–16] and the prevalence of IgE sensitization to other aeroallergens is very low in Cartagena [11], sIgE levels against these two mite extracts were determined by indirect ELISA in all individuals as previously described [17]. sIgE levels above 0.110 optical densities (ODs) corresponding to a mean OD of 5 nonallergic controls + 3 SD were considered positive. Atopy was defined as positive sIgE to at least one of the two mite extracts tested.

Genotyping

Six single-nucleotide polymorphisms (SNPs) in *ADAM33*, rs528557 (S2), rs574174 (ST+7), rs2280091 (T1), s2280090 (T2), rs543749 (V_1) and rs2787094 (V4) were chosen for genotyping based on previously published studies identifying putative risk alleles [3]. DNA was extracted from peripheral blood samples using standard protocols [18]. Genotyping of the 6 SNPs was performed by the TaqMan-probe-based, 5′-nuclease allelic discrimination assay [19] on the 7900HT Sequence Detection System (Applied Biosystems-ABI, Foster City, Calif., USA). TaqMan® validated assays and master mix were manufactured by Applied Biosystems (Applied Biosystems). The PCR was done in a 5-μl volume using a universal master mix, 4 predesigned and validated TaqMan® assays for the SNPs rs528557, rs2280091, rs2280090 and rs2787094 (ABI catalogue No.: C_601719_20, C1_5969370_10, C_15969380_10 and C_11201381_1, respectively) and 2 customized quality-controlled TaqMan® assays for SNPs rs574174 and rs543749. The thermal cycling conditions were: 95°C for 10 min followed by 40 cycles of 95°C for 15 s/60°C for 1 min and an extension step of 60°C for 5 min. Nontemplate negative controls and genotyping-positive controls were included in each genotyping plate. Automatic calling was done with a quality value above 99%.

Genotyping Quality Control

Approximately 8% of samples were repeated for quality control with an average error rate of 0.2%. Mendelian inconsistencies were identified using Sib-Pair (1.00a17) with 0.3% discordant genotype pairs in the family dataset. Genotypes were made miss-

ing as appropriate for members of nuclear families involved, which rendered 14/1980 genotypes (0.7%) missing for further analyses.

Statistical Methods

The outcome variables were asthma, tIgE and sIgE. Asthma status was considered as a dichotomous trait (i.e., presence/absence) and sIgE against *B. tropicalis* and *D. pteronyssinus* as continuous variables. tIgE was log transformed (log tIgE), percentiles were calculated based on all available subjects with existing tIgE measurements (n = 1,178) and log tIgE 90th percentile (3.12 UI/ml) was used as dependent variable (above/below the 90th percentile) with two categories denoted as 'high' and 'low' tIgE levels, respectively. Comparisons of the demographic characteristics among cases and controls and among cases and family probands were done by means of Student's t test and the χ^2 test as needed. Departure from Hardy-Weinberg equilibrium proportions of each SNP was tested among controls and parents. Fisher's exact test was used to compare allelic frequencies between the cases and controls and between individuals with high and low tIgE levels. Logistic regression was performed to model the effect of genotype on asthma status and tIgE levels (high or low) including age and sex as covariates; linear regression was used to analyze sIgE levels as outcome variable under the same model. Linkage disequilibrium measures of allelic association D′ [20] were done according to Haploview (http://www.broad.mit.edu/mpg/haploview). Haplotypic frequencies in asthmatics, controls and in individuals with high and low tIgE levels were estimated using the expectation-maximization algorithm of Excoffier and Slatkin [21] using ARLEQUIN software [22]. In the haplotypic analysis, each individual contributed two haplotypes (one from each chromosome) to the analyses and the frequency and estimated counts of each haplotype were then assessed against all others using standard procedures for odds ratio (OR) and confidence interval (CI) calculations. All calculations were done with Statistical Package for the Social Sciences software (SPSS 16 for Windows, SPSS Inc., Chicago, Ill., USA). In the family trios, tests of linkage and association between each SNP and their haplotypes, and asthma, high tIgE and sIgE against *B. tropicalis* and *D. pteronyssinus* were conducted using the family-based association test (FBAT Version 2.0.2, available at: http://www.biostat.harvard.edu/~fbat/default.html) [23]. Association tests between the 6 polymorphisms and the phenotypes were performed under an additive model for alleles and a genotype model for genotypes. For haplotype analysis, haplotype frequencies were obtained by an expectation-maximization algorithm, which maximizes the likelihood of phased haplotype frequencies based on all observed genotypes in the nuclear family and haplotype permutation tests were performed to determine association of haplotypes with the analyzed traits.

Correction by Population Stratification

A panel of 52 ancestry-informative markers was also genotyped in the total sample. These markers showed large differences in frequency between the parental populations and were used to control for genetic structure due to recent admixture as previously described [24]. To test for association of the SNPs in the candidate genes within the phenotypes correcting by genetic population structure, we used the program ADMIXMAP (available at http://www.lshtm.ac.uk/eu/genetics/index.html#admix). This is a general-purpose program for modeling population admixture with genotype and phenotype data, based on a combination of Bayesian and classical methods. The statistical algorithms used in this program have been described in detail previously [25, 26] and its efficacy to adjust association analysis of candidate genes by individual admixture has been demonstrated for other traits [27]. Briefly, the program fits a hierarchical model for the distribution of admixture proportions in the population, the admixture proportions of each parental gamete, and the ancestry of the gene copies at each locus. The variation between three states of ancestry on chromosomes of mixed descent is modeled as the outcome of three independent Poisson arrival processes with the sum of the intensities of the arrival processes specified by the user. Allele and haplotype frequencies are estimated by combining information from unadmixed and admixed population samples. A generalized linear model is specified for the relation of the trait to individual admixture and other covariates. The model is specified as a Bayesian full-probability model, in which haplotypes, ancestry states at each locus, gamete admixture proportions, and population level parameters are specified as 'missing data'. The posterior distribution of these data, given the observed data, is generated by Markov chain Monte Carlo simulation and inference with the parameters of the regression model is based on posterior distribution. In large samples, the posterior means and 95% central posterior intervals are asymptotically equivalent to maximum-likelihood estimates and 95% confidence intervals. Score tests for allelic association with the trait, conditional on individual admixture and any other covariates are performed by testing a coefficient b parameter for the effect of the allele or haplotype under study (coded as 0, 1, or 2 copies) in a regression model. For each SNP, a positive score value indicates association of the trait with the allele being tested. To test the null hypothesis, i.e. that b = 0, the score (gradient of the log likelihood) and the observed information (curvature of the log likelihood) at b = 0 are calculated by averaging over the posterior distribution of the missing data (the haplotypes and individual admixture values). The score test correctly allows for uncertainty about haplotype assignments and estimation of individual admixture proportions because it is based on the likelihood of the observed data as a function of parameter b that is being tested. The ratio of observed to complete information in the score test can be interpreted as a measure of the efficiency of the analysis, compared to a study design in which haplotypes have been observed directly and individual admixture proportions measured without error. Where an allele or haplotype is found to show significant association with the trait, it is possible to estimate the size of the effect of that allele by fitting a model in which the allele or haplotype (coded for each individual as 0, 1, or 2 copies) is included as an explanatory variable in the regression model. Inference is then based upon the posterior distribution of the regression coefficient. An approximation to the maximum-likelihood estimate of the effect can be obtained from the score test by dividing the score by the observed information. In large samples, this is asymptotically equivalent to computing the maximum-likelihood estimate directly. For this analysis, the Cartagena population was modeled as formed by admixture between three subpopulations: European, Native American and West African. Score tests for allelic/haplotype association with the traits, conditional on individual admixture and age and sex as covariates were constructed by testing coefficient b for the effect of the allele/haplotype under study (coded as 0, 1, or 2 copies) in a regression model. An approximation to the maximum-likeli-

Table 1. Demographic and laboratory characteristics of the subjects in the case-control and family-based datasets from Cartagena, Colombia

Characteristics	Cases (n = 429)	Controls (n = 401)	p value
Age, means ± SD, years	36.15 ± 18.32	34.98 ± 17.8	0.348
Male gender, n	163 (38.0)	173 (43.1)	0.317
Atopy, n	396 (83.3)	12 (3.04)	<0.001
log serum tIgE, means ± SD, IU/ml	2.7 ± 0.41	1.9 ± 0.53	<0.001
High serum tIgE, 90th percentile, n	53 (12.4)	2 (0.5)	<0.001
Serum *B. tropicalis*-sIgE, means ± SD, OD	0.88 ± 1.09	0.094 ± 0.42	<0.001
Serum *D. pteronyssinus*-sIgE, means ± SD, OD	0.57 ± 0.82	0.096 ± 0.61	<0.001
	Offsprings (n = 116)	Founders (n = 232)	
Age, means ± SD, years	17.2 ± 9.9	46.5 ± 11.1	
Male gender, n	58 (50.0)	116 (50.0)	
Atopy, n	91 (78.4)	114 (49.1)	
log serum tIgE, means ± SD, IU/ml	2.7 ± 0.50	2.32 ± 0.53	
High serum tIgE , 90th percentile, n	24 (20.6)	4 (1.7)	
Serum *B. tropicalis*-sIgE, means ± SD, OD	0.95 ± 1.13	0.26 ± 0.50	
Serum *D. pteronyssinus*-sIgE, means ± SD, OD	0.53 ± 0.80	0.20 ± 0.33	

Figures in parentheses are percentages.

hood estimate of the effect size was obtained from the score test by dividing the score by the observed information. A corrected p value below 0.05 (p_c) was considered statistically significant. In addition, Bonferroni's correction for multiple tests was applied to p_c values in the case-control analyses and to raw p values in the family-based analyses; therefore, after 6 independent tests, a single test would be significant with a p value < 0.008.

Results

Demographic and clinical characteristics of the case-control dataset and the 116 family trios are shown in table 1. Cases and controls had similar age and sex distributions (p = 0.3 and p = 0.3, respectively). log tIgE and sIgE were significantly higher in cases than in controls ($p < 0.001$). Most asthmatic individuals were atopic in both the case group (83.3%) and the offspring group (78.4%). Asthmatic offsprings were younger than cases ($p < 0.001$). Levels of tIgE (mean ± SD: 2.7 ± 0.41 vs. 2.7 ± 0.50, p = 0.7) sIgE against *B. tropicalis* (0.88 ± 1.09 vs. 0.95 ± 1.13, p = 0.5) and sIgE against *D. pteronyssinus* (mean ± SD: 0.57 ± 0.82 vs. 0.53 ± 0.80, p = 0.6) were similar among these two groups.

The 6 SNPs selected for genotyping are shown in figure 1a. They are located from exon 19 to the 3′-UTR region and showed variable pairwise D′ values ranging from 0.10 to 0.94 in the total sample (n = 1,178). As shown in figure 1b, two haplotype blocks were found using the algorithm based on the solid spine of LD; no blocks were found using the algorithm of Gabriel et al. [28]. Allelic and genotypic frequencies in cases and controls, and Hardy-Weinberg equilibrium p values in controls are presented in online supplementary table 1 (www.karger.com/doi/10.1159/000260081). All SNPs were in Hardy-Weinberg equilibrium in both controls and founders (data not shown). Single SNP association tests with asthma and high total IgE levels (90th percentile) in this dataset are displayed in table 2, which also shows the results of the allelic association with both traits obtained in a regression model conditioned on individual admixture and including age and sex covariates. TT genotype of ST+7 was associated with asthma (OR: 2.42; 95% CI: 0.99–5.9, p = 0.05), but the significance disappeared when correcting for multiple testing.

Results of the family-based association tests of the single polymorphisms with all the phenotypes are displayed in tables 3 and online supplementary table 2. In this da-

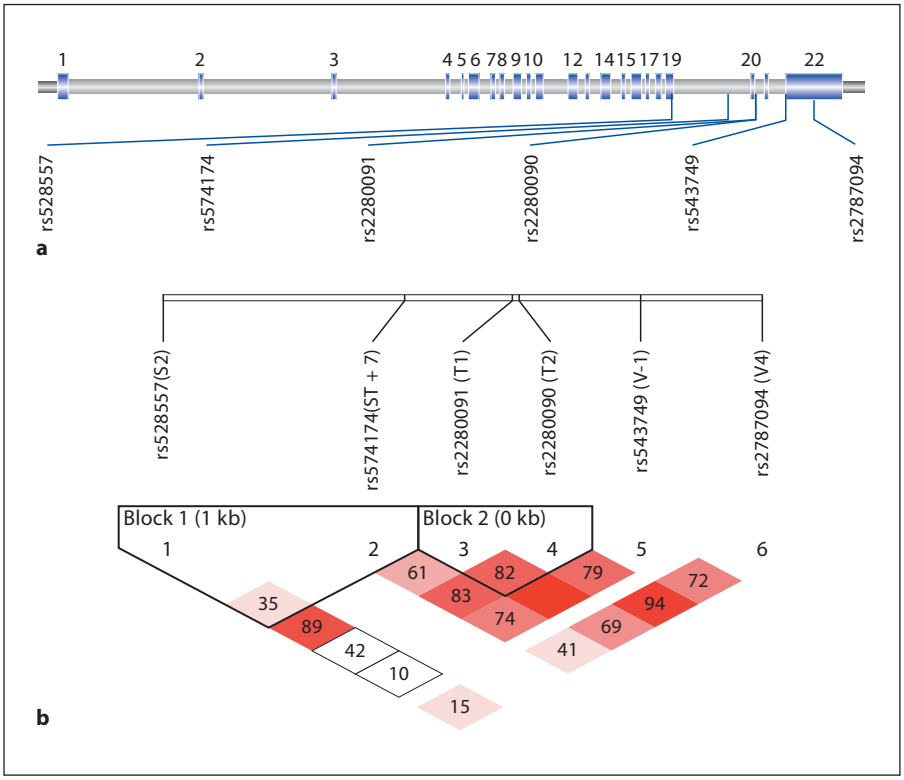

Fig. 1. a Schematic overview of the exon intron distribution of the *ADAM33* gene located on chromosome 20p13 and position of the six SNPs selected for genotyping. The chromosomal position of these SNPs (reference contig NT 011387.8) is as follows: rs2787094 (V4): 3597161 (C/G), rs543749 (V_1): 3597679 (G/T), rs2280090 (T2): 3598205 (A/G), rs2280091 (T1): 3598234 (A/G), rs574174 (ST+7): 3598694 (C/T) and, rs528557 (S2): 3599742 (C/G). A = Adenine; C = cytosine; G = guanine; T = thymine; rs = database SNP accession number. **b** The 2,581-bp region containing haplotype blocks in the *ADAM33* gene, as defined by solid spine of LD. The numbers in each box correspond to the pair-wise linkage disequilibrium coefficient D′ between the respective SNPs. All the subjects included in the case-control groups and families were analyzed together (n = 1,178). The SNPs are ordered according to their position in the gene (see **a**).

taset, we replicated the association of TT genotype of ST+7 with asthma (Z score: 2.414, p = 0.01); this genotype was also significantly associated with high tIgE levels (Z score: 2.546, p = 0.01), high levels of sIgE against B. *tropicalis* (Z score: 2.188, p = 0.02) and *D. pteronyssinus* (Z score: 2.414, p = 0.01). CC genotype of V4 was associated with high levels of sIgE against *B. tropicalis* (Z score: 2.089, p = 0.03); AA genotype of T2 was associated with asthma (Z score: 2.065, p = 0.03), high tIgE (Z score: 2.268, p = 0.02) and high levels of sIgE against *D. pteronyssinus* (Z score: 2.065, p = 0.03); GG genotype of T1 was associated with high tIgE levels (Z score: 2.041, p = 0.04). These associations did not remain after correction for multiple comparisons.

Estimated frequencies of haplotypes in the total sample from Cartagena are shown in online supplementary table 3. The results of the haplotype association analyses with asthma and high tIgE levels in the case-control and the families are shown in table 4. To estimate the haplotypic association controlling by stratification, ADMIXMAP modeled the unobserved haplotypes, conditional on the observed unordered genotypes. Score tests for association of haplotypes with the trait were obtained using a regression model adjusting for individual admixture and other covariates. No associations between haplotypes and asthma or high IgE levels were found in the case-control group; however, H4 (GCAGGG) was significantly associated with asthma in the family-based analysis (Z score: –2.049, p = 0.04, permutation test p value = 0.037).

Discussion

In this study, we analyzed 6 polymorphisms and their haplotypes in *ADAM33* to investigate their association with asthma and tIgE and sIgE levels against 2 domestic mites using 2 independent approaches in a population of the Caribbean Coast of Colombia. H4 haplotype (GCAGGG in **S2/ST+7/T1/T2/V-1/V4**) was significantly associated with asthma in the family based analysis.

The results of association studies between asthma and *ADAM33* haplotypes have been contradictory. A H4-comparable 6-SNP haplotype G**CC**G**TCCC** in S1/**S2**/ST+4/**ST+7/T1/T2/V-1/V4** (common SNPs are shown in bold with alleles corresponding to the opposite strand) was not associated with asthma in Dutch, African American, White and Hispanic populations [29]; similar re-

Table 2. Association between the *ADAM33* polymorphisms and asthma and high tIgE levels (90th percentile) in the case-control analysis in the population of Cartagena, Colombia

	Asthma				High tIgE levels			
	OR	CI	p^1	p_c^2	OR	CI	p^1	p_c^2
rs528557 (S2)								
C	1.07	0.88–1.3	0.47	0.20	0.97	0.64–1.47	0.88	0.76
CC	1.09	0.82–1.4	0.54		1.14	0.64–2.01	0.66	
CG	0.96	0.73–1.3	0.80		0.85	0.48–1.47	0.56	
GG	0.90	0.61–1.3	0.62		1.1	0.48–2.50	0.82	
rs574174 (ST+7)								
C	0.9	0.72–1.2	0.62	0.65	0.82	0.50–1.38	0.43	0.54
CC	1.01	0.75–1.3	0.94		1.4	0.80–2.5	0.23	
CT	0.88	0.65–1.2	0.41		0.66	0.37–1.2	0.16	
TT	2.42	0.99–5.9	0.05		1.7	0.22–12.8	0.61	
rs2280091 (T1)								
A	1.2	0.89–1.7	0.20	0.27	1.63	0.72–3.89	0.21	0.25
AA	1.19	0.84–1.7	0.32		0.68	0.30–1.53	0.35	
AG	0.88	0.61–1.3	0.52		1.3	0.59–2.9	0.50	
GG	0.49	0.15–1.7	0.26		1	0.0–0.0	0.99	
rs2280090 (T2)								
A	1.02	0.74–1.4	0.88	0.78	0.62	0.26–1.41	0.22	0.26
AA	1.16	0.35–3.8	0.81		1	0.0–0.0	0.99	
AG	1.01	0.69–1.4	0.95		0.75	0.33–1.71	0.49	
GG	0.97	0.68–1.4	0.89		1.5	0.65–3.3	0.36	
rs543749 (V_1)								
G	1.05	0.83–1.3	0.68	0.50	0.97	0.60–1.6	0.91	0.97
GG	1.01	0.76–1.35	0.89		0.88	0.50–1.5	0.67	
GT	1.05	0.78–1.40	0.79		1.184	0.67–2.1	0.56	
TT	0.74	0.38–1.4	0.36		0.79	0.19–3.40	0.76	
rs2787094 (V4)								
C	0.98	0.79–1.2	0.90	0.43	1.01	0.64–1.59	0.97	0.63
CC	1.02	0.629–1.6	0.94		1.28	0.52–3.13	0.58	
CG	1.07	0.80–1.4	0.64		1.24	0.68–2.24	0.48	
GG	1.06	0.79–1.4	0.68		1.12	0.63–1.9	0.70	

[1] Allelic and genotypic association corrected by age and sex.
[2] Allelic association corrected by age, sex and population structure.

sults were observed with Ht1 (S1/**T1/V-1/T4**) and BHR in Korean asthmatics [30]. The 3 SNP-haplotype TCC (in T1/V-1/V4**)** was not related to asthma in Puerto Ricans and Mexicans [31], but positive associations were found with asthma and asthma + BHR in UK and US populations [3]. In Australians, there was also a significant global haplotypic association with asthma (p = 0.0002) and disease severity (p = 0.0001), driven by the combination of the SNPs V_1 and ST+7 [32]. The remarkable dissimilarities in haplotype diversity and frequency do not allow comparisons with some studies [32, 33] and can explain the discrepancy of results across populations. The protective effect of the H4 haplotype on asthma in the family analysis indicates that *ADAM33* can be considered as genetic factor influencing the risk of developing the disease in this particular population. However, additional research should be done to define a finer haplotype structure of the complete gene and its boundary regions in this population.

We corrected for multiple comparisons, and although there were reasons for suspecting this gene would be linked to the asthma risk, the SNPs can be considered as highly correlated and, therefore, a comprehensive correction would be overly conservative. Furthermore, we analyzed the association of this gene with asthma and allergic response, correcting for differences in the admixture proportions between asthmatic and controls (population structure), which confers additional consistency to our findings.

Table 3. Family-based association tests (FBATs) of *ADAM33* polymorphisms and asthma, high tIgE levels (90th percentile) and IgE levels against *B. tropicalis* and *D. pteronyssinus* in a sample from Cartagena, Colombia

Allele/ genotype	Informative families, n	Asthma		High tIgE levels (90th percentile)	
		Z score	FBAT p value	Z score	FBAT p value
rs528557 (S2)					
C	70	0.209	0.834	0.246	0.805
G	70	–0.209	0.834	–0.246	0.805
CC	60	–0.135	0.892	0.000	1.000
CG	70	0.478	0.632	0.280	0.779
GG	32	–0.583	0.560	–0.466	0.641
rs574174 (ST+7)					
C	39	–0.590	0.555	–0.563	0.573
T	39	0.590	0.555	0.563	0.573
CC	37	0.421	0.6737	0.510	0.610
CT	39	–1.441	0.1495	–1.565	0.117
TT	9	2.414	0.0157	2.546	0.010
rs2280091 (T1)					
A	33	0.324	0.745	0.651	0.515
G	33	–0.324	0.745	–0.651	0.515
AA	33	7.938	0.329	1.429	0.1531
AG	33	–8.250	0.117	–2.100	0.0356
GG	5	0.938	0.070	2.041	0.0412
rs2280090 (T2)					
A	37	–0.309	0.757	–0.630	0.528
G	37	0.309	0.757	0.630	0.528
AA	6	2.065	0.038	2.268	0.0233
AG	37	–1.808	0.070	–2.292	0.0218
GG	36	1.103	0.270	1.525	0.1271
rs543749 (V_1)					
G	55	0.728	0.466	0.282	0.777
T	55	–0.728	0.466	–0.282	0.777
GG	51	0.796	0.426	0.554	0.579
GT	55	–0.674	0.500	–0.734	0.463
TT	17	–0.135	0.892	0.442	0.658
rs2787094 (V4)					
C	66	1.725	0.084	1.728	0.084
G	66	–1.725	0.084	–1.728	0.084
CC	26	1.746	0.080	1.761	0.781
CG	66	0.000	1.000	–0.198	0.843
GG	60	–1.079	0.280	–1.013	0.311

Several reasons can explain the lack of associations of single SNPs observed in this study. One of them is a low power to detect risks in this complex disease. Considering a prevalence of asthma of 0.10 [9], the case-control sample used in this analysis has a power of 0.80 to detect an OR of 1.5 with an α-level of 0.05 and a minor allele frequency of 0.10 (http://pngu.mgh.harvard.edu/~purcell/cgi-bin/cc2.cgi). Nevertheless, if we consider a lower level of significance to result from the correction by multiple testing, the power of this sample decreases remarkably. Another reason is the kind of phenotypes analyzed. Given the contributory role of *ADAM3* in bronchial remodeling, it would be worth analyzing its association with traits other than pulmonary function. Several results have indicated a link between *ADAM33* and this parameter in both infant and adult populations. The involvement of several variants with diverse effects has been described, a common phenomenon in complex diseases. For instance, Simpson et al. [34] analyzed 17 SNPs in *ADAM33* and their relationship with pulmonary function decline in a cohort of children from Manchester evaluated at ages of 3 and 5 years. They found a significant association between S1, V4 and ST+5 with this phenotype at 5 years; and F+1 was associated with it at 3 and 5 years. Similarly, in a cohort of 200 Dutch Caucasian asthmatics followed over 20 years, S2, T1 and T2 were associated with accelerated lung function decline represented by the annual decline in forced expiratory volume in 1 s [35]. Moreover, in a cohort of 1,390 subjects from the Netherlands followed for 25 years, S_2, Q-1, S_1 was significantly associated with an accelerated decline in forced expiratory volume in 1 s; in the same population, chronic obstructive pulmonary disease was significantly associated with F+1, S_1, S_2, and T_2 [36]. The small number of patients with pulmonary function measurements in our dataset precluded this analysis with a reasonably powered sample. It is also possible that *ADAM33* has a more relevant role in nonatopic asthma, which implicates local lung mechanisms. In two independent samples of German children, Schedel et al. [37] reported an association between nonatopic asthma and allele A of S1 (OR 1.53; 95% IC: 1.01–2.31, p = 0.04) as well as allele G of V4 (OR 1.44; 95% IC: 1.03–2.01, p = 0.03). Similarly, allele T of M+1 conferred protection for nonatopic asthma (OR 0.60; 95% IC: 0.40–0.91, p = 0.01) in this study. The sample evaluated in our study was mainly comprised of atopic asthmatics with a low proportion of nonatopic asthmatics, which did not allow us to test the association between *ADAM33* and this type of asthma.

On the other hand, it is also important to consider the genetic diversity of the populations reflected in differences in the occurrence of polymorphisms and their allelic frequencies. For instance, the allelic distributions of the SNPs S2 and V4 in our population are different from other populations (online suppl. table 4). Also, our sample differs in terms of genetic structure from some populations in which positive associations between *ADAM33*

Table 4. Association between haplotypes in the *ADAM33* gene and asthma and high tIgE levels (90th percentile) in the family-based dataset from Cartagena, Colombia

Haplotype	Cases and controls (n = 429/n = 401)								Family-based associated analysis (n = 428)					
	asthma				high IgE levels (90th percentile)				asthma			high IgE levels (90th percentile)		
	OR	CI	p	p_c[1]	OR	CI	p	p_c[1]	Z score	p	p^2	Z score	p	p^2
H1	0.95	0.76–1.19	0.64	0.88	1.0	0.62–1.60	0.99	0.81	0.734	0.46	0.94	0.542	0.58	0.83
H2	1.14	0.87–1.48	0.33	0.22	1.07	0.62–1.82	0.79	0.71	1.017	0.26	0.42	0.629	0.52	0.79
H3	0.99	0.70–1.40	0.95	0.75	1.36	0.70–2.57	0.32	0.30	0.122	0.90	0.99	0.093	0.92	0.98
H4	1.19	0.81–1.75	0.34	0.41	1.10	0.51–2.33	0.78	0.84	–2.049	0.040	0.037	–1.728	0.083	0.073
H5	0.81	0.51–1.28	0.34	0.42	0.86	0.30–2.28	0.75	0.70	–			–		
H6	0.62	0.36–1.07	0.06	0.09	0.67	0.17–2.27	0.50	0.60	–			–		
H7	0.62	0-36–1.07	0.06	0.09	0.92	0.28–2.71	0.87	0.70	1.430	0.152	0.094	1.742	0.081	0.058
H8	0.80	0.45–1.43	0.42	0.81	0.86	0.30–2.28	0.75	0.90	–0.577	0.56	0.49	0.000	1.0	0.75

[1] Corrected by age, sex and population structure.
[2] Permutation test p value.

and asthma and related phenotypes were found [38]. They included Australians [32], Dutch Caucasians [35], European-Americans and African Americans [29], Koreans [30], Icelanders and British individuals [39], and Japanese [40, 41].

Our findings concerning individual SNPs agree with some investigations that found negative results; for example, in a case-control and family study in Mexican American and Puerto Rican populations [31], where the relationship between the SNPs T1, V_1, V4 and S1 and asthma, asthma severity and bronchial responsiveness was analyzed [31]. They also agree with an investigation in children from two German populations analyzing the association between 10 SNPs (including those analyzed in the present study) and asthma as well as BHR [37] and with an evaluation of 13 SNPs and their association with asthma in Icelandic and British populations [39].

In summary, with highly stringent criteria, we found no associations between alleles or genotypes of 6 polymorphisms of *ADAM33* and asthma or IgE; nevertheless, at the haplotype level, *ADAM33* can be considered as a risk factor for asthma in this population of Colombia. The weak associations observed here suggest either a small effect of this gene in the pathogenesis of asthma and allergy, as usually described in complex diseases, or the existence of causal alleles different from those we tested. Further studies are needed to elucidate the variants responsible for the significant result at the haplotype level.

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