

Association of a disintegrin and metalloprotease 33 (*ADAM33*) gene with asthma in ethnically diverse populations

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Background: Asthma is a complex genetic disease characterized by reversible intermittent airway obstruction and respiratory symptoms primarily caused by acute and chronic bronchial inflammation. Recently, a gene potentially involved in airway remodeling, a disintegrin and metalloprotease 33 (*ADAM33*), was implicated in asthma susceptibility.

Objective: We sought to determine whether polymorphisms in *ADAM33* are associated with asthma or closely related phenotypes in 4 different asthma populations.

Methods: Eight single nucleotide polymorphisms (SNPs) were evaluated in the 3' portion of *ADAM33* in 4 unique asthma populations (African American, US white, US Hispanic, and Dutch white). These SNPs were previously reported to be associated with asthma in white populations from the United States and United Kingdom.

Results: Significant associations were observed with at least one SNP and asthma in each population ($P = .0009-.04$). Related phenotypes that included total serum IgE levels and skin test responsiveness were also associated ($P = .003-.05$). However, no single SNP was associated across all populations. Additionally, haplotype analysis revealed that no single haplotype accounted for asthma susceptibility risk, although potential risk haplotypes existed within some of the populations.

Conclusion: Replication of the original *ADAM33* findings in these 4 additional asthma populations suggests that this gene (and perhaps others that interact with it) is important in the development and pathogenesis of asthma. (*J Allergy Clin Immunol* 2003;112:717-22.)

Key words: Asthma, *ADAM33*, association, haplotype

Asthma is a common respiratory disease characterized by intermittent airway obstruction and respiratory symptoms caused by acute and chronic bronchial inflammation. The development of asthma appears to be determined by the interaction between host susceptibility and

Abbreviations used

ADAM: A disintegrin and metalloprotease
BHR: Bronchial hyperresponsiveness
SNP: Single nucleotide polymorphism

a variety of environmental exposures. The results from numerous genetic studies indicate that the contributions of multiple genes are likely to be involved in the susceptibility and progression of asthma and other closely related disorders (eg, Xu et al¹).

A recent study of 460 white families from the United States and United Kingdom used a positional cloning approach to identify a candidate region on chromosome 20p for susceptibility to asthma, bronchial hyperresponsiveness (BHR), and variations in total and specific IgE levels.² Further investigation by this group identified the putative candidate gene as a disintegrin and metalloprotease 33 (*ADAM33*). Multiple single nucleotide polymorphisms (SNPs) within *ADAM33* were significantly associated with asthma and BHR for either the US population, the UK population, or both populations combined. The majority of associated SNPs were located in the 3' half of the gene, extending from exon Q to the last exon, exon V.

ADAM33 is one member of the ADAM family, a subfamily of transmembrane metalloproteinases that have both a disintegrin and metalloproteinase domain, although these are not functional in all ADAMs. Much of the interest of the recently discovered ADAMs is due to their functions in biologic processes, including fertilization, neurogenesis, myogenesis, embryonic TGF- α release, and inflammatory response. ADAMs have been implicated in critical functions, such as cell-cell and cell-matrix interactions, cell migration, cell adhesion, and signal transduction. Although there is some variation between the existing family members, characteristic features of ADAM proteins include a signal sequence and the following domains: pro, metalloprotease (including a zinc binding sequence), disintegrin, cysteine-rich, epidermal growth factor, transmembrane, and cytoplasmic tail domains. One function of ADAM proteins is the shedding of the extracellular portion of specific cytokines and growth factors, leading to the soluble forms of these proteins.

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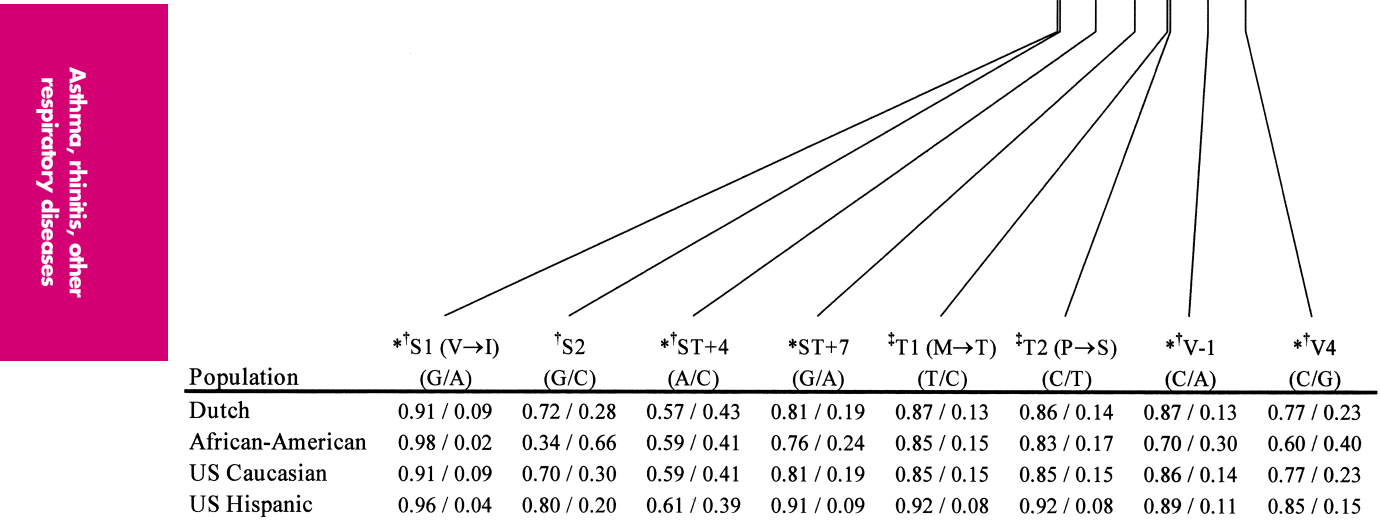


FIG 1. Genomic structure of *ADAM33* and the location of the 8 SNPs genotyped. The 22 exons are shown as boxes and designated by the letters A through V. The SNPs genotyped and the allele frequencies in all 4 asthma case-control populations are shown in the table below. SNPs that were previously associated² are indicated: *combined UK and US populations, †UK population only, ‡US population only.

To further characterize the contribution of *ADAM33* to asthma susceptibility and related phenotypes, we have evaluated 8 SNPs that were previously reported to be associated in either the US or UK populations² and the resulting haplotypes in 4 case-control asthma populations: African American, US white, US Hispanic, and Dutch white. These populations have been useful resources recruited to study the genetics of asthma and allergy, including candidate gene analysis.³⁻⁶ Our primary hypothesis for this study was that variation in the *ADAM33* gene contributes to susceptibility to asthma or, as a secondary hypothesis, to closely related asthma and allergy phenotypes.

METHODS

Asthma cases in the African American, US white, and US Hispanic populations were collected by using criteria established as part of the Collaborative Study on the Genetics of Asthma.⁷ Control subjects for this group had no history of asthma and no first-degree relatives with asthma. Dutch probands with clinical asthma were originally characterized between 1962 and 1975.^{8,9} Between 1990 and 1998, 200 probands and their spouses, children, and available grandchildren were studied. The probands and spouses represent an appropriate unrelated sample for association studies because they are of similar ethnicity, age, and environmental exposures, reducing the possible confounding effect of age on changes in BHR, total serum IgE levels, and atopy. This study was approved by the Medical Ethics Committee at the University of Groningen, and both studies were approved by the Institutional Review Board at Wake Forest University School of Medicine. Written informed consent for all adults and written parental assent for children were obtained from all participants.

The original Dutch probands were compared with the Dutch spouses without BHR (PC₂₀ >32 mg/mL histamine) to analyze the asthma phenotype. Skin test responsiveness was measured in adult

subjects by means of intracutaneous skin testing with 16 (Dutch) common aeroallergens, and results were considered positive if the maximum wheal diameter was 5 mm or larger. In the Collaborative Study on the Genetics of Asthma (18 allergens) skin prick testing was used, and results were considered positive if the maximum wheal diameter was 3 mm or larger.

To further characterize the role of *ADAM33* with asthma susceptibility,² we evaluated 8 SNPs (S1, S2, ST+4, ST+7, T1, T2, V-1, and V4) in the 3' portion of the gene in 4 asthma populations. Genotyping was performed by using the MassARRAY SNP genotyping system (Sequenom, Inc). PCR and extension primers were designed by using SpectroDesigner software (Sequenom, Inc), and reactions were performed according to the manufacturer's instructions. All 8 SNPs investigated were in Hardy-Weinberg equilibrium. Each SNP was analyzed by comparing differences in genotype frequencies between the case and control groups stratified by population. χ^2 tests were performed by using codominant, dominant, and recessive genetic models. Fisher exact tests were performed when less than 5 individuals were in any one genotype group. Haplotypes were determined by using the program PHASE.¹⁰ Similar results were observed with a different program (haplo.score) for evaluating haplotypes.¹¹ Total serum IgE values were logarithm transformed to approximate a normal distribution and analyzed as a quantitative trait. Differences between groups were tested with ANOVA, *t* tests, and multiple regression.

RESULTS

Some variability in allele frequency was observed among the 4 populations (Fig 1).² For asthma susceptibility, significant associations were observed to at least one of the 8 SNPs within each population, but no single SNP was consistently associated with a specific asthma phenotype across all of the groups (Table I). The most significant associations were observed in the Dutch asthma population with SNPs ST+7 and V4 by using a codominant model (*P* = .0093 and *P* = .0009, respective-

TABLE I. Genotype frequencies for *ADAM33* SNPs and asthma susceptibility

	Dutch		African American		White		Hispanic	
	Patients	Control subjects	Patients	Control subjects	Patients	Control subjects	Patients	Control subjects
S1 (V→I)	n = 153	n = 124	n = 160	n = 256	n = 219	n = 225	n = 112	n = 126
GG	0.86	0.83	0.96	0.97	0.82	0.81	0.91	0.92
GA	0.14	0.15	0.04	0.03	0.17	0.19	0.09	0.08
AA	0.00	0.02	0.00	0.00	0.01	0.00	0.00	0.00
	<i>P</i> = NS		<i>P</i> = NS		<i>P</i> = NS		<i>P</i> = NS	
S2	n = 150	n = 123	n = 157	n = 249	n = 218	n = 225	n = 111	n = 125
GG	0.57	0.55	0.10	0.12	0.54	0.44	0.59	0.72
GC	0.37	0.34	0.39	0.49	0.39	0.46	0.35	0.23
CC	0.07	0.11	0.50	0.39	0.08	0.10	0.05	0.05
	<i>P</i> = NS		<i>P</i> = .03*		<i>P</i> = .052		<i>P</i> = .04†	
ST+4	n = 173	n = 127	n = 151	n = 257	n = 211	n = 215	n = 111	n = 117
AA	0.20	0.19	0.42	0.33	0.36	0.33	0.34	0.37
AC	0.51	0.44	0.45	0.47	0.46	0.50	0.50	0.53
CC	0.29	0.37	0.13	0.20	0.18	0.17	0.16	0.10
	<i>P</i> = NS		<i>P</i> = NS		<i>P</i> = NS		<i>P</i> = NS	
ST+7	n = 180	n = 132	n = 159	n = 258	n = 217	n = 229	n = 109	n = 126
GG	0.72	0.64	0.54	0.58	0.65	0.64	0.82	0.82
GA	0.27	0.29	0.41	0.37	0.33	0.29	0.17	0.18
AA	0.01	0.08	0.05	0.05	0.01	0.07	0.01	0.00
	<i>P</i> = .0093‡, <i>P</i> = .0051*		<i>P</i> = NS		<i>P</i> = .017‡		<i>P</i> = NS	
T1 (M→T)	n = 179	n = 133	n = 159	n = 261	n = 216	n = 229	n = 107	n = 120
TT	0.74	0.74	0.67	0.75	0.77	0.68	0.64	0.66
TC	0.24	0.23	0.29	0.22	0.21	0.31	0.34	0.32
CC	0.02	0.03	0.04	0.03	0.02	0.01	0.03	0.03
	<i>P</i> = NS		<i>P</i> = NS		<i>P</i> = .03†		<i>P</i> = .06†	
T2 (P→S)	n = 180	n = 133	n = 160	n = 263	n = 216	n = 229	n = 111	n = 127
CC	0.74	0.74	0.66	0.71	0.77	0.67	0.80	0.90
CT	0.24	0.23	0.29	0.26	0.21	0.32	0.19	0.09
TT	0.02	0.03	0.04	0.03	0.02	0.01	0.01	0.01
	<i>P</i> = NS		<i>P</i> = NS		<i>P</i> = .02†		<i>P</i> = .04†	
V-1	n = 180	n = 132	n = 159	n = 260	n = 219	n = 226	n = 112	n = 127
CC	0.79	0.77	0.47	0.50	0.74	0.75	0.79	0.81
CA	0.20	0.20	0.46	0.42	0.25	0.21	0.21	0.17
AA	0.01	0.03	0.07	0.08	0.01	0.04	0.01	0.02
	<i>P</i> = NS		<i>P</i> = NS		<i>P</i> = NS		<i>P</i> = NS	
V4	n = 179	n = 131	n = 161	n = 265	n = 220	n = 229	n = 113	n = 127
CC	0.54	0.72	0.37	0.32	0.60	0.58	0.66	0.76
CG	0.42	0.22	0.47	0.52	0.33	0.38	0.30	0.22
GG	0.04	0.06	0.16	0.15	0.06	0.05	0.04	0.02
	<i>P</i> = .0009‡, <i>P</i> = .0012†		<i>P</i> = NS		<i>P</i> = NS		<i>P</i> = NS	

NS, Not significant.

*Common allele is dominant.

†Common allele is recessive.

‡Cotodominant model.

ly). Associations with asthma were also observed in the African American (S2, *P* = .03), US white (ST+7, *P* = .017; T1, *P* = .03; T2, *P* = .02), and US Hispanic (S2, *P* = .04; T2, *P* = .04) populations.

In addition to asthma, associated phenotypes, such as skin test responsiveness and variations in total serum IgE levels, were evaluated (Table II). As with asthma, at least one SNP was observed to be significantly associated with one of these 2 phenotypes in each population; however, no single SNP was consistently associated across populations. Significant associations were observed for total serum IgE levels in both the US white and Hispanic populations (*P* = .0033-.032, Table II) centered around SNPs T1 and T2.

Construction of haplotypes revealed significantly different haplotype frequencies between the patients and control subjects in all but the Hispanic population (Table III). However, no single haplotype appeared to differ consistently between patients and control subjects across all 4 populations. Not surprisingly, more haplotypes were observed in the African American population (*n* = 30) than in the other 3 populations. The most significant differences were observed with 2 haplotypes in the Dutch population. The haplotype GGCGTCCG was more common in the probands (0.14) compared with that in the spouses without BHR (0.065; *P* < .005), whereas the haplotype GGCATC-CC was more common in the spouses without BHR (0.086) compared with that in the probands (0.038; *P* < .01).

Asthma, rhinitis, other
respiratory diseases

TABLE II. Asthma-related phenotypes associated with SNPs in *ADAM33*

Population	Phenotype	S1 (V→1)	S2	ST+4	ST+7	T1 (M→T)	T2 (P→S)	V-1	V4
Dutch	Positive ST	$P = .037^*$	$P = .014^\dagger$		$P = .046^*$			$P = .050^*$	
	Total serum IgE								
African American	Positive ST			$P = .022^\ddagger$					$P = .017^\ddagger$
	Total serum IgE								
US white	Positive ST		$P = .031^*$			$P = .0033^*$	$P = .0037^*$		
	Total serum IgE								
US Hispanic	Positive ST			$P = .032^*$		$P = .0099^*$	$P = .0092^*$	$P = .020^\ddagger$	
	Total serum IgE								

ST, Skin test.
*Common allele is recessive.
†Codominant model.
‡Common allele is dominant.

TABLE III. Haplotype frequencies of *ADAM33* SNPs in ethnically diverse populations

	Dutch		African American		White		Hispanic	
	Patients (n = 197)	Control subjects (n = 146)	Patients (n = 166)	Control subjects (n = 269)	Patients (n = 227)	Control subjects (n = 244)	Patients (n = 116)	Control subjects (n = 129)
ACAATCAC	—	0.003	—	—	0.007	0.002	0.004	0.031
ACAATCAG	0.071	0.082	0.018	0.015	0.084	0.086	0.039	0.008
AGAATCAG	—	—	—	—	—	0.004	—	—
GCAATCAC	—	—	0.084	0.119	—	—	0.004	0.019
GCAATCAG	—	—	0.069*	0.024	—	0.002	—	—
GCAATCCC	—	—	—	0.009	—	—	—	—
GCAGCCCG	—	—	0.012	0.020	—	—	—	—
GCAGCTAC	—	—	—	0.004	—	—	—	—
GCAGCTCC	0.117	0.123	0.145	0.104	0.119	0.156	0.095	0.058
GCAGCTCG	—	—	0.018	0.011	0.002	—	—	—
GCAGTCAC	—	—	0.003	0.002	—	—	—	—
GCAGTCAG	—	—	0.003	—	—	—	—	—
GCAGTCCC	—	—	0.105	0.097	0.002	0.029	0.004	—
GCAGTCCG	—	—	0.030	0.033	—	—	0.009	0.008
GCCATCAC	0.020	0.027	0.045	0.039	0.031	0.029	0.026	—
GCCATCAG	0.005	0.010	0.012	0.013	0.004	0.012	0.009	0.008
GCCGCTCC	—	—	0.003	—	—	0.002	—	—
GCCGTCAG	—	—	0.006	0.009	—	—	—	—
GCCGTCCC	0.005	—	0.015	0.015	0.011	0.016	0.013	0.027
GCCGTCCG	0.010	—	0.133	0.115	—	—	0.017	—
GGAATCAG	—	—	—	0.002	—	—	—	—
GGAGCTCC	0.005	0.007	—	—	0.004	—	—	—
GGAGCTCG	—	—	0.006	—	—	—	—	—
GGAGTCAC	—	—	0.018	0.002	—	—	—	0.012
GGAGTCAG	—	—	0.033	0.035	—	—	—	—
GGAGTCCC	0.353	0.366	0.090	0.063	0.37†	0.309	0.431	0.488
GGAGTCCG	—	—	0.003	0.030	—	—	—	—
GGAGTTAG	—	—	0.003	—	—	—	—	—
GGCATCCC	0.038‡	0.086	0.018	0.013	0.051	0.064	0.017	0.031
GGCGTCC	0.003	—	—	—	—	—	—	—
GGCGTCAC	—	—	—	—	—	0.002	0.004	—
GGCGTCAG	—	—	—	—	—	—	0.022	0.023
GGCGTCCC	0.228	0.229	0.066	0.086	0.183	0.166	0.211	0.209
GGCGTCCG	0.14*	0.065	0.048‡	0.099	0.132	0.119	0.091	0.078
GGCGTTAC	—	—	0.009	0.035	—	0.002	0.004	—
GGCGTTCC	0.003	—	0.003	0.006	—	—	—	—

* $P < .005$.
† $P < .05$.
‡ $P < .01$.

DISCUSSION

The report of *ADAM33* as a positionally cloned gene for asthma² represents an important development in understanding susceptibility to asthma, allergy, and closely related phenotypes.^{12,13} Thus, it is necessary to evaluate the importance of this observation by replicating it in different populations and further delineating the role of this gene in asthma. The purpose of this study was to evaluate variation in *ADAM33* in 3 ethnic groups in the United States (African American, white, and Hispanic), as well as a well-defined northern European (Dutch) population. We chose to evaluate a subset of the SNPs previously described, with a focus on those that were associated with asthma in the US or UK populations.² This led to the assessment of 8 SNPs in the 3' portion of this gene, which provides new information indicating the importance of *ADAM33* in asthma susceptibility in these 4 populations. Significant associations were observed with asthma susceptibility, as well as with one or more allergy- or asthma-associated phenotypes, in all 4 populations, indicating this gene's potential role as a general asthma susceptibility locus.

Our results support the previously reported associations for *ADAM33*.² We observed significant associations with several SNPs in the 3' portion of *ADAM33* but were unable to determine the primary SNP involved. In general, the results between the 2 white populations (US and Dutch) are similar, but there are some interesting differences (Table I). Both populations showed nonsignificant results for 4 of the SNPs (S1, S2, ST+4, and V-1), less significant results in the US population for 2 SNPs (T1 and T2), and significant results for 1 SNP (ST+7). Different results were observed for the V4 SNP, which was significant in the Dutch white subjects ($P = .0009$) but not significant in the US white subjects. For T1 and T2, which show borderline significance in the US white subjects, the frequencies in the Dutch and US white asthmatic patients are similar, but the frequencies differ in the control subjects, causing the differences in the overall results. Also, for V4, which is significant in the Dutch white subjects but not significant in the US white subjects, the frequency of the CC genotype is similar in both sets of subjects with asthma (0.54 in Dutch vs 0.60 in US white subjects); however, the control frequencies differ substantially (0.72 in Dutch vs 0.58 in US white subjects). The frequency of the V4 common allele is similar for the Dutch patients, US white patients, and US white control subjects (0.75, 0.77, and 0.77, respectively). However, the allele frequency in the Dutch control subjects is quite different (0.83), causing the significant association. These values also differ with the data for the UK population previously reported (0.84 in patients and 0.75 in control subjects).² These data suggest that another nearby SNP might be responsible for the observed phenotypic effects, and, because of the relatively small population sizes in the studies to date, the existing data vary.

Although single SNP associations were observed in each population, no single SNP was associated in all populations, suggesting that the causative polymorphism

has not yet been identified. This suggests one of several possibilities. First, a different and undefined SNP or haplotype is in linkage disequilibrium with the SNPs associated in each population. Thus, the difference between populations might be due to differences in linkage disequilibrium in each population. Second, different SNPs might be important in specific ethnic groups or populations. This could be due to variation in the genetic background or simply an effect of different environmental exposures (eg, active and passive smoking and allergen exposure) specific to each group. In either case replication studies in larger and different asthma populations are required to identify the genetic relationship between *ADAM33* and disease.

Further support for the likelihood that an unidentified SNP or haplotype is responsible can be observed in the haplotype analysis. Unless a different and unique risk haplotype exists for each of the 4 populations studied (which is unlikely, given the limited ethnic diversity between US and Dutch white subjects), the haplotype data suggest that the causative SNP or haplotype has not yet been identified. For example, in the Dutch population the GGCGTCCG haplotype was more than twice as common in probands as in unaffected spouses (0.142 vs 0.065, respectively). In the population, however, this haplotype was more common in the control subjects than in the patients (0.099 vs 0.048, respectively). Likewise, the risk haplotype in the US white subjects, GGAGTCC, is more common in patients but contradicted by data in the US Hispanic and Dutch populations. Evaluation of additional polymorphisms in *ADAM33* is required to identify the contributing SNP or haplotype.

Three of the SNPs examined created coding changes (S1, Val to Ile; T1, Met to Thr; and T2, Pro to Ser). These SNPs were only weakly associated with asthma susceptibility in either the white (T1) or the white and Hispanic (T2) populations and with skin test responsiveness in the Dutch (S1) population. T1 and T2 were also strongly associated with total serum IgE levels in the white and Hispanic populations, indicating that these SNPs might be functionally relevant. Because all of these populations were ascertained for asthma, the frequency of allergy in these populations is also high. Thus, because of the close association of allergic factors in the development and expression of asthma, one would expect overlap in these phenotypic characteristics.¹⁴ In fact, we have previously demonstrated the strong correlation between total serum IgE levels and BHR in the Dutch population.⁹ If these SNPs do not have a functional effect, it is possible that an additional SNP or haplotype in linkage disequilibrium with the examined SNPs might be responsible for a functional change in *ADAM33*. Alternatively, a known or novel gene nearby might affect asthma susceptibility.

ADAM33 was recently identified as an asthma susceptibility gene after using a positional cloning approach in a sib-pair population from the United States and United Kingdom.² Interestingly, chromosome 20p13 is not a locus that has been detected in many other genome screens for asthma- or atopy-related phenotypes. In the 4

populations examined in this report, linkage to this region was observed only in the US white population (lod score = 1.07), as well as in the combined US populations, when conditional analysis was performed for linkage to chromosome 6p.¹ The lack of linkage in the other populations suggests that *ADAM33* confers a modest effect but has a fairly large attributable risk, as previously described for complex disease genes in which common alleles contribute to susceptibility.¹⁵

To date, more than 30 ADAMs have been identified.¹⁶ ADAM proteins have been shown or predicted to have various functions, and some are known as sheddases (eg, ADAM17/TACE, ADAM10, and ADAM9) because of their ability to release the extracellular portion of membrane proteins. *ADAM33* has been characterized in the mouse^{17,18} and human subjects.^{2,18} Phylogenetic analysis indicates that in human subjects *ADAM33* is most similar to *ADAM8*, *ADAM12*, *ADAM15*, and *ADAM19*.¹⁸ Expression data suggest that *ADAM33* is expressed in multiple tissues, including lung fibroblasts, bronchial smooth muscle, and whole lung.^{2,18}

It is biologically plausible that *ADAM33* is relevant to the development of asthma because cell-cell and cell-matrix interactions are essential for the development and maintenance of an organism; likewise, extracellular matrix proteolysis is an important contributor to tissue remodeling. With asthma, airway remodeling might ultimately lead to significant airway obstruction or, perhaps, more severe BHR. Moreover, tissue integrity is an important aspect of the airway wall as a barrier to inhaled particles, and as such, ADAMs might play an important role in this process, given their potential ability to perform both adhesion and proteolytic functions.

The combined associations with asthma susceptibility and asthma-associated phenotypes (ie, skin test positivity and total serum IgE levels) in addition to the previously described expression data strongly support the role of *ADAM33* as one of the susceptibility genes for asthma or closely associated risk phenotypes. Further population and functional studies are necessary to further define its specific role and interactions with other genes important in these disorders.

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