

EDITORIAL

***ADAM33* gene: confirming a gene without linkage**

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The development of asthma is determined by a complex interaction between host susceptibility and a variety of environmental exposures. These interactions provide the signals that eventually lead to intermittent airways obstruction and the respiratory symptoms caused by acute and chronic bronchial inflammation. To date, numerous family- and population-based studies have indicated that multiple genes are likely to be involved in the development of asthma, as well as its intermediate phenotypes. However, only recently have genes for asthma susceptibility been identified by positional cloning, a technique that has been successful in monogenic diseases.

A recent study of 460 Caucasian families from the US and UK used positional cloning to identify the *ADAM33* gene on chromosome 20p for susceptibility to asthma, bronchial hyper-responsiveness, and variations in total and specific IgE levels [1]. Multiple single-nucleotide polymorphisms (SNPs) within *ADAM33* were significantly associated with asthma and BHR in a US or UK population, or with these two 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.

The excitement of discovering a gene for a complex disease is somewhat offset by the anticipation for others to replicate the original findings. So far, three articles have recently been published, two confirming and one refuting the relevance of the *ADAM33* gene in the development of asthma [2–4]. The article by Werner et al. [4] in this issue of Clinical and Experimental Allergy has added to the credibility that *ADAM33* is a genuine factor in the pathogenesis of asthma. In this article, 15 SNPs from *ADAM33* that were associated in the original study were tested in two German populations: (i) an asthma family study consisting of 171 families ascertained through two children with asthma (89% physician-diagnosed), and (ii) a case-control population consisting of 547 individuals. Forty-eight of the latter population had asthma, whereas 91 had no BHR, and only 14 subjects had asthma and BHR. Significant associations for asthma were observed with SNPs F+1, ST+4 and ST+5 in the German families and with ST+7 in the case-control group. These results are very exciting in that they replicate the finding that SNPs in *ADAM33* are associated with asthma susceptibility.

We have also replicated the findings from the original report in three ethnically diverse populations [2], further supporting this gene's potential role as a general asthma susceptibility locus. Eight SNPs (also associated in the original study) were examined in Dutch and US Caucasian, African-American and Hispanic populations. The SNPs associated with asthma susceptibility were S2 (African-Americans and Hispanics), ST+7 (Dutch and US Caucasians), T1 (US Caucasians and Hispanics), T2 (US Caucasians

and Hispanics) and V4 (Dutch Caucasians). While we observed a significant association with at least one SNP in each population, no single variant was consistently associated with asthma and its intermediate phenotypes in all populations.

All SNPs genotyped in our study were also examined in the article by Werner et al. [4] in this issue of the journal. There is an overlap of significant association with only one of the SNPs tested – ST+7. This SNP was also associated in the original study, but only in the combined US and UK group. The ST+7 locus lies in an intron between exons 19 and 20. While there is no obvious functional effect of this polymorphism, it is possible that regulatory sequences located within this intron affect transcriptional efficiency or transcript stability, leading to a flux in *ADAM33* mRNA levels, which could eventually lead to disease susceptibility. Another explanation for the lack of consistency of associated SNPs is the differences in linkage disequilibrium (LD) among the populations. This would imply that a nearby SNP, or possibly even a different gene, that is in LD with the SNPs tested is responsible for the functional effect that leads to asthma. This remains to be determined in future analyses.

It is of interest that only the original report demonstrated significant evidence for linkage to chromosome 20p, albeit a lod score of 1.07 was observed in this region in the US Caucasian population [5]. This suggests that genes with a significant contribution to disease may be missed by relying solely on family-based linkage studies. Of the three replication studies now available, two also found a significant association of *ADAM33* SNPs with asthma [2, 4], while a study by Lind et al. [3] in Mexican and Puerto Rican populations did not confirm the association. A lack of association may occur due to differences in phenotypes, ascertainment or size of the populations under study, different environmental exposures (e.g. smoking), variability in LD between populations, or actual differences between the SNPs responsible in each population. Both Van Eerdewegh et al. [1] and Lind et al. [3] used the phenotype 'doctor diagnosis of asthma with additional current use of asthma medication' in their association analyses. The association improved in the study of Van Eerdewegh when both asthma and BHR had to be present. This might explain the lack of association in the study of Lind et al. [3], since BHR was not assessed. However, BHR was also absent in many asthmatics with a doctor diagnosis in the study by Werner et al. [4] and they did observe a significant association. Furthermore, the controls in the study of Lind et al. [3] were not objectively tested for the presence or absence of asthma and atopy. Thus, one cannot exclude a bias in the results due to confounding of atopy and/or asymptomatic BHR in the controls, both known to be present in a substantial proportion of the general population [6, 7].

While the sample sizes for each individual population may be considered relatively small, the emerging picture is that ADAM33 contributes to asthma in multiple ethnic groups.

While the asthma-associated SNPs differ somewhat between studies, an examination of allele frequency differences between cases and controls in each group suggests a potential trend in most populations. There is a consistent pattern for a subset of SNPs that have been genotyped in most populations: a preponderance of the minor allele in cases at the ST+4 locus, and the major allele in controls at the ST+7 locus (Fig. 1). While this representation is not a standard method of interpreting data from various studies, it suggests that there is a trend that seems to be present in most of the populations. Interestingly, the largest difference between cases and controls is observed in the US population from the original study. This difference may be due to the fact that the controls in this study were considered 'hyper-normal', meaning they did not have asthma and were skin-test negative for four common allergens [1]. However, this is unlikely to explain the observation completely, since the controls in our population did not have BHR or atopy as well.

To understand the role of ADAM33 in asthma, it is useful to examine the putative function as well as the expression pattern of the gene product. Preliminary characterization of mouse and human ADAM33 protein suggests that it has an active metalloprotease domain, which is not true of all ADAM family members. ADAMs function in a variety of biological processes, including fertilization, neurogenesis, myogenesis, embryogenic TNF- α release and the inflammatory responses [8]. ADAMs have been implicated in critical functions such as cell-cell and cell-matrix interactions, cell migration, cell adhesion and signal transduction. While there is some variation between the ~ 30 existing family members, ADAM proteins in general contain a highly conserved

domain structure. One particularly interesting function of some ADAM proteins is the 'shedding' of the extracellular portion of specific cytokines and growth factors, leading to the soluble forms of these proteins. A primary example of this is ADAM17, which was originally shown to be responsible for the shedding of TNF- α [9].

A recent study [10] showed that *ADAM33* mRNA was significantly expressed in smooth muscle containing organs and minimally in immune organs, suggesting a role for ADAM33 in remodelling rather than in immune responses. In asthmatics, it was expressed in subepithelial fibroblasts and smooth muscle, yet not in respiratory epithelium. Tissue integrity is also an important aspect of the airway wall as a barrier to inhaled particles and, as such, ADAMs may play an important role in this process, given their potential ability to perform both adhesion and proteolytic functions. However, this should be an indirect effect on epithelial cells, given its lack of expression in epithelial cells. A putative role in airway wall remodelling may also explain the loss of lung function observed in a genotypic subset with the S2 polymorphism in our Dutch asthma population (unpublished data). The significantly excess decline in lung function existed in the Dutch Caucasian asthma individuals who were homozygous for the C allele of the S2 polymorphism (submitted data). While the sample size was small for the CC group ($n = 9$), these data suggest that ADAM33 may not only be responsible for susceptibility to asthma but also for progression of the disease.

To date, the exact role of ADAM33 as a disease susceptibility and disease-modifying gene is unclear. The article of Werner et al. [4] contributes to the insight that the gene is important for the development of asthma. Further studies on the exact function and substrate utilization are important to unravel its mechanisms of action in the development and progression of asthma.

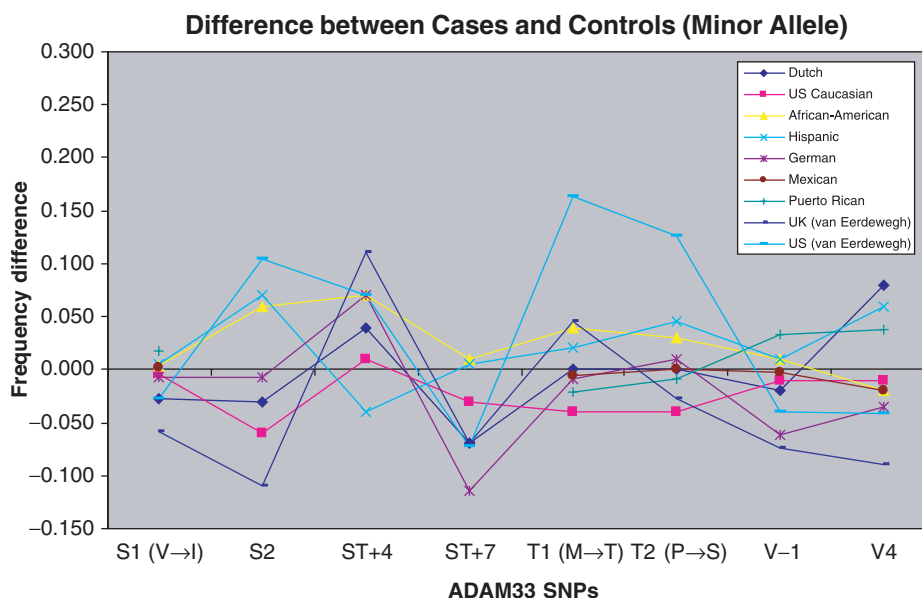


Fig. 1. Differences in frequency of single-nucleotide polymorphisms (SNPs) in cases and controls in different studies.

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