The Internet Journal of Asthma, Allergy and Immunology
Volume 5 Number 2

Asthma Gene Detection with Association Studies

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Citation

G Pesola, R Gundavarapu, S Abdul-Waheed, S Haider. *Asthma Gene Detection with Association Studies*. The Internet Journal of Asthma, Allergy and Immunology. 2006 Volume 5 Number 2.

Abstract

Genetic association studies are currently one of the most common ways of attempting to characterize complex diseases felt to have a genetic background. The rationale behind association studies using asthma as the disease will be reviewed.

INTRODUCTION

Asthma is a syndromic disease characterized by episodic cough and wheezing secondary to airway narrowing that usually follows from airway inflammation. It is felt to be a gene-environment interaction ([1]). This means that it is possible for an individual to have the correct genetic make-up for developing asthma and yet never manifest disease if the environmental exposure is not present to promote the clinical syndrome. In general, genetic predisposition is a necessary but not sufficient cause for the development of asthma. An argument can be made that almost all diseases, except possibly autosomal dominant genetic diseases such as Huntington's disease as an example, are gene-environment interactions ([2]). Inheriting the Huntington gene would be a rare necessary and sufficient cause for the development of Huntington's disease and would not require environmental input.

Most simple genetic diseases caused by one genetic locus have undoubtedly been found and it is felt that asthma is a complex syndrome associated with multiple predisposing genes ([3]). The most popular current approach to identifying potential genes associated with asthma is to identify a “candidate gene” whose function suggests that there could be a role in asthma ([4]). Then an asthma phenotype is defined by the investigators, asthmatics and nonasthmatic controls are enrolled in a study and genotyped, and a comparison is made between cases and controls to determine if there is a difference in haplotypes or single nucleotide polymorphisms. If there is a significant difference that can be replicated, it suggests that a given gene polymorphism or haplotype is associated with asthma. Over 118 genes have been associated with asthma or atopy in at least one clinical study ([3]). At least 54 of these 118 genes have been replicated in one or more follow-up studies by different investigators suggesting a possible association between asthma or atopy and the specific gene.

CASE-CONTROL STUDY APPROACH

It is generally felt that the most powerful approach to detecting small genetic effects that might occur with complex diseases such as asthma is by testing a few select candidate genes with the case-control association study ([5]). These studies usually require less than one thousand subjects and often less than 500 to detect differences if they exist ([5]). The study is easy to employ and starts by obtaining a homogeneous population of a well defined phenotype to test a candidate gene. An equal number of cases of nonasthmatic controls are then picked randomly from the same population as the cases. Ideally, the environment of the cases and controls is very similar. The distribution of candidate gene SNPs and/or haplotypes are compared between cases and controls. If there are significant differences between the cases relative to the controls in any of these SNP/haplotypes, this is a positive association study. The preliminary finding needs to be corroborated at least once by independent investigators to validate the results.

The true difficulty in the detection of disease even with this study design approach relates to the need for good control of a dual exposure. One exposure is clear cut, the genetic predisposition that is present at birth. Although this could change in the near future, currently this is assumed to be unalterable and precedes disease. The second exposure is the environment that is presumed to interact with a given set of genes to produce the clinical expression of asthma. Making this even more difficult, it is probable that different sets of genes interact with different environments. Sorting this out

by selecting controls with a similar environment as cases will be difficult; but might be a more ideal approach to exposure management in this type of study.

In the next decade, genome-wide association studies will be able to evaluate over 100,000 genetic markers at one time ([5]). This theoretically will facilitate a faster identification of the genetic determinants of complex diseases. A difficult problem is to decide which of the greater than 4 million genetic variants should be evaluated in each screen.

FALSE POSITIVE RESULTS

Problems inherent in the candidate gene approach to determining the genetic underpinnings of asthma include false positive results that occur at least 5% of the time due to chance alone. In theory, false positives can be eliminated by repeating the study. If the study cannot be replicated it may have been a false positive. Before assuming lack of replication in a second study, however, it should be remembered that one reason for lack of replication is studying the same gene with a slightly different phenotype. Comparison between studies should always be done with the same phenotype and this has been a major problem between studies. A second reason for obtaining a false positive association is secondary to population stratification. In this instance, the control population is not the ideal source population from which the cases originated. This can result in genetic differences between cases and controls suggesting a false asthma gene association. The association only appears to be true because the case and control populations were different to begin with and had nothing to do with differences related to asthma. The apparent association can be eliminated by further studies using controls that come from the same population as the cases. Therefore, if cases come from a specific ethnic group, then controls have to originate from the same ethnic group. A third source of error in obtaining false positive associations relates to comparing many candidates genes or many polymorphisms on one gene in the same study. This results in the multiple comparisons false positive rate that can be partly controlled by statistical means. At the same time, it must be remembered that the best way to safely eliminate a false positive result of this nature is to replicate the study. If there is no association in a second or third study, it is unlikely the initial association was correct. Finally, systematic genotyping errors can occur leading to false positive results ([6]). Retyping possible errors is one solution if suspected. Also, if cases and controls are in Hardy-Weinberg equilibrium the errors in genotyping will often not be in equilibrium suggesting an error in genotyping.

FALSE NEGATIVE RESULTS

Asthma is a gene-environment interaction. Therefore, it is likely that many subjects have a genetic background that is consistent with asthma in some environments but not others. These potential asthmatics may sit in a group of controls who are sampled and do not reveal a difference consistent with an association with asthma cases due to the varied living conditions of the cases compared to the controls. In other words, if the cases and controls come from slightly different environmental backgrounds, it is possible that the asthma cases have nondetectable genetic differences compared to controls because some of the controls would have been cases if they had been exposed to the same environment as the cases. Tight control of environmental factors along with the asthma phenotype might better facilitate the detection of asthma genes in clinical studies ([7]).

It is estimated that there are numerous negative association studies with many candidate genes that have never been reported simply because they are negative ([4]). Unfortunately, these candidate gene studies will have to be published at least once in the future to make other investigators aware. It is possible some of these negative studies are false negative and did not have a strong asthma phenotype, or lack of environmental control was a confounder between cases and controls.

As noted above, at least 54 of 118 positive association studies for asthma or atopy were replicated at least a second time. Many initially positive studies are not replicated and may simply be false positives. A third possibility is an initial true positive with a subsequent replication study that is a false negative secondary to utilizing a slightly different phenotype or possibly environmental confounding. Therefore, asthma gene association studies that will stand the test of time undoubtedly will need a series of positive studies to solidify an association.

Last, a power analysis should be done a priori to get a sense of the size of the study needed to detect a difference if one exists. Given an allele frequency of 25% and 94 cases and 94 controls, there would be only an 80% power to detect a 2-fold difference between cases and controls if one existed ([8]). Most studies are small with low allele frequencies and undoubtedly miss some associations secondary to lack of power.

CONCLUSION

Unfortunately, complex diseases such as asthma do not have necessary or sufficient causes that facilitate the easy detection of etiology. Instead, most of these diseases are multifaceted gene-environment interactions that are difficult to sort out.

Currently, the case-control association study is the most reliable way to detect small genetic differences between asthmatics and nonasthmatics. Due to the relative ease of implementation, fast results, and multiple genetic backgrounds that are felt to be associated with asthma, this approach will continue to be popular in the near future and will be amplified with the genome-wide search. In order to increase the likelihood of finding real genetic associations, attention to detail in avoiding both false positive and false negative results are needed. Independent replication will always be required to confirm an initial positive association.

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