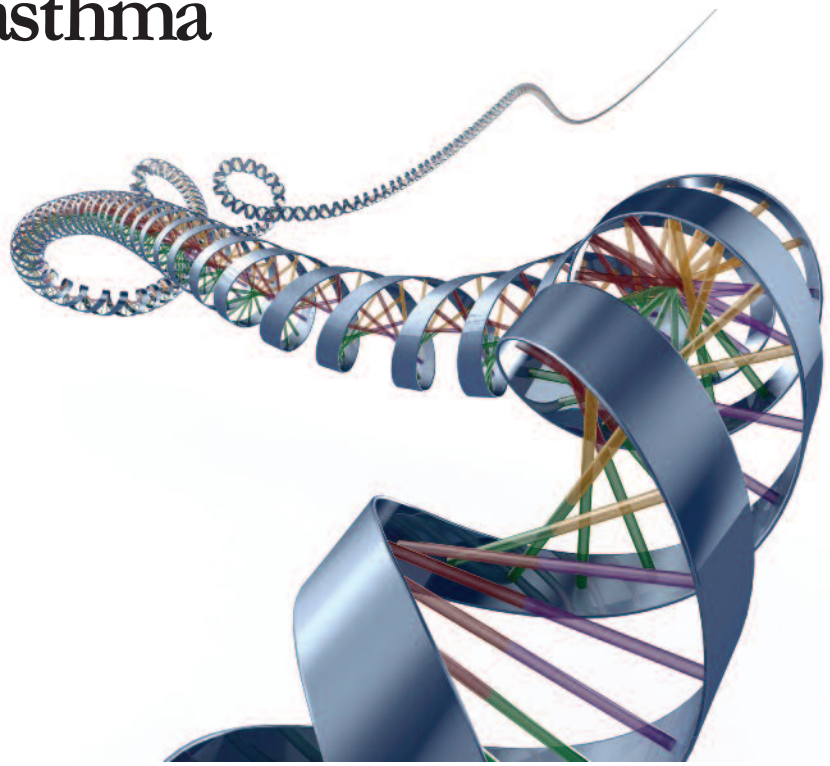
Gene–environment interactions in asthma: Lessons for the development of new drugs for asthma

Adnan Custovic and Angela Simpson
The University of Manchester



Introduction

Asthma is among the most common chronic diseases in developed countries.[1] Despite availability of effective drugs, a number of asthmatic patients who adhere to their treatment continue having troublesome symptoms.[2] For example, in one study of asthmatic children, 55% did not respond to either fluticasone or montelukast.[3] Very little has changed in asthma therapy in the last few decades, with inhaled corticosteroids and beta-2 agonists remaining the cornerstone of treatment.[4] Few genuinely novel therapeutic agents have entered clinical practice and the cure for asthma seems as elusive as ever.

Asthma endotypes

Currently, there is no universally accepted definition of asthma and no consensus on the pathophysiology underlying the disease. It is likely that asthma is not a single disease, but a collection of diseases presenting as a syndrome,[5-8] with symptoms such as wheezing being a common feature of a number of different disease mechanisms[9] with unique pathophysiology and environmental and genetic associates.[6,8] Two recent reports have proposed that "asthma syndrome" should be divided into distinct entities assigned as "asthma endotypes."[10,11] An endotype is a subtype of a condition, which is defined by a distinct pathophysiological mechanism.[11] Unless we are able to clearly define true asthma endotypes, it will not be possible to identify novel, endotype-specific, therapeutic targets, as any signal will constantly be diluted by phenotypic heterogeneity. This conceptual framework may also explain why the responses to currently available treatments vary considerably[3], with a proportion of "asthmatic" patients having relatively poor response. If asthma is an umbrella diagnosis which comprises multiple diseases with distinct mechanisms, then it is unlikely that these different diseases would respond to the same therapeutic agents.

Different approaches to identification and classification of asthma "endotypes" have been used. One approach is to reach a consensus among experts; for example, the ERS Task Force consensus statement on "Preschool Wheeze" defined phenotypes of preschool wheezing disorders using the terms "episodic viral wheeze" and "multiple-trigger wheeze,"[12] but has recognized that there is a large overlap between these phenotypes, and that patients can move from one phenotype to another.

Another approach is to use longitudinally collected data to assign phenotypes based on temporal patterns of wheeze (e.g. using answers to a single repeated question, such as phenotyping described in the Tucson Children's Respiratory Study which defined Transient Early Wheezing, Late-Onset Wheezing and Persistent Wheezing).[13] As a refinement of this approach, investigators in ALSPAC birth cohort performed a latent class analysis on a dataset collected annually over a 7-year period and identified six childhood wheezing phenotypes.[6] Several recent publications have demonstrated

the utility of "unbiased" statistical clustering approach in multidimensional data to identify different asthma phenotypes.[7,8,14] We conducted a Principal Component Analysis using answers to multiple questions and identified five syndromes of coexisting symptoms in preschool children.[8] Unsupervised hierarchical cluster analysis suggested five distinct clinical phenotypes of adult severe asthma in the US Severe Asthma Research Program,[7] and a similar approach in Leicester, UK, identified two clusters of refractory asthma characterized by discordance between symptom expression and eosinophilic airway inflammation.[5] Phenotypic heterogeneity is not a problem only in asthma, and similarly applies to atopy (e.g., we have recently reported that atopy may include several endotypes which differ in their association with symptomatic disease,[15] providing possible explanation for previous observations that a proportion of "atopic" individuals have no objective markers of allergic disease).[16] It appears clear that we need fundamentally new approaches to phenotyping to reduce the error-prone subjective assessments.

Genetics of asthma

Twin and family studies suggest a strong genetic component of asthma.[17] However, genetic studies have produced heterogeneous results with little replication.[18] Furthermore, "precise" replication (i.e., the same association of the same genetic variant with the same phenotype) is rare.[19] In current literature, replication usually refers to the finding of any association between the gene and any asthma-related phenotype (e.g. atopy or lung function). Frequently, different single nucleotide polymorphisms (SNPs) are associated with different disease phenotypes in different populations. In some instances, contradictory findings are accepted as replication (e.g. when the same genetic variant is associated with increased risk of asthma in one population and decreased risk in another).[18] Results of studies on the genetics of asthma have so far had limited impact on diagnosis, prediction of response to currently available treatments or the development of novel therapeutic targets.

Asthma and environment

There has been a sharp increase in asthma prevalence since the 1960s, emphasizing the important role of environmental exposures in asthma development.[1] Numerous environmental factors have been implicated, and a number of environmental changes have occurred in parallel with the increase in asthma (including changes in microbial exposure, diet, exercise, childhood immunizations, family size, childcare arrangements and housing design). However, similar to genetic studies, there is a heterogeneity in the results of studies which investigated the role of environmental exposures. For example, cat ownership has been shown to increase the risk,[20] decrease the risk[21] or have no effect.[22] Similarly, attendance to nursery school in early life in different studies was associated with either increase in risk, protection from asthma[24] or had no effect.[25] The inconsistencies are also reflected in intervention studies;[26] for example, a primary prevention study investigating the effect of allergen avoidance has produced counter-intuitive findings of an increased incidence in mite allergy despite markedly reduced mite allergen exposure.[27] Despite decades of extensive study, we still cannot give advice on ways to modify the environment to minimize the risk of asthma.

Gene-environment interactions

A body of evidence suggests that asthma arises as a consequence of environmental factors, increasing the risk in genetically susceptible individuals, and that this is mediated through gene-environment interactions.[28] We have demonstrated an interaction between environmental exposure to endotoxin and polymorphism in gene encoding the pattern recognition receptor for endotoxin (CD14) in the development of wheeze, atopy and eczema.[29]

Definitions

Allele: one of two or more forms of a gene or a genetic locus (generally a group of genes); differing alleles can result in different observable phenotypic traits

Endotype: a subtype of a condition, which is defined by a distinct functional or pathobiological mechanism; distinct from a phenotype

Filaggrin: a filament-associated protein that binds to keratin fibers in epithelial cells

Genotype: the genetic makeup of a cell, organism or individual (i.e. the specific allele makeup of an individual)

Phenotype: an organism's observable characteristics or traits, such as its morphology, development, biochemical or physiological properties, behavior and products of behavior

Single-nucleotide polymorphism (SNP, pronounced snip): a DNA sequence variation occurring when a single nucleotide — A, T, C or G — in the genome (or other shared sequence) differs between members of a biological species or paired chromosomes in an individual

Zygosity: the similarity of alleles for a trait in an organism; if both alleles are the same, the organism is homozygous for the trait; if both alleles are different, the organism is heterozygous for that trait

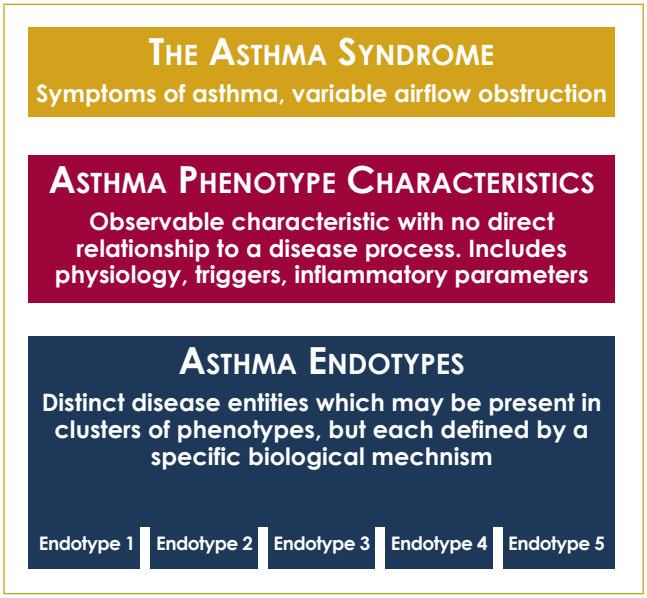
Source: Wikipedia

Similar to other environmental factors, in some studies exposure to endotoxin was found be protective,[30,31] in others it increased the risk,[32] while some reported no effect on asthma/allergy.[33] The genetic association between a functional variant in CD14 promoter (CD14/-159)[34] and asthma/allergy has been investigated in many populations; like many other genes, some studies found no association,[35] some found the C allele,[34] whereas others found the T allele to increase the risk.[36] We have shown that high endotoxin exposure is protective against atopy and eczema, but only among children with a particular genotype (C allele homozygotes).[29] These results may explain the disparities in association studies of this SNP in different settings,[37] indicating that in genetic association studies carried out in communities exposed to high level of endotoxin (e.g., farming), the T allele would be the "risk allele,"[36] in communities with low endotoxin exposure, the same allele (T) would be protective,[34] while in communities with moderate exposures this genotype would not be associated with the outcome.[35]

In two independent, population-based, birth cohorts from the UK and the US, we have recently demonstrated that the association between day-care attendance in early life and asthma development during childhood appears dependent on a genetic variant in the TLR2 gene.[38] Attending day-care was protective, but only among children carrying the T allele for TLR2/–16934; while among AA homozygotes, the association tended to be in the opposite direction. The true relevance of the TLR2 gene was only uncovered when the relevant environmental exposure was identified and taken into account (i.e., we found no association between genotype and asthma before we explored TLR2 gene interaction with day-care attendance). Similarly, day-care attendance appeared protective against asthma in the analysis of the whole population,[24] concealing the fact that, in a subgroup of children (AA homozygotes for TLR2/–16934), it actually increased the risk of disease.[38] The apparent protective effect in the whole population arose from the fact that children in whom day-care attendance was protective outnumbered those in whom it increased the risk by a factor of ~4:1.[38] In a similar example, we have recently reported that cat ownership increases the risk of early-life eczema, but only in children with filaggrin loss-of-function variants (~10% of the population), and not among those without.[39] These examples emphasise the point: if genotypes are studied in isolation, irrespective of the size of the population studied, associations can and would be missed.

Genetic variants and response to drugs in asthma

Pharmacogenetics (a study of genetic variations that give rise to differing response to drugs) can be considered a relatively simple example of gene-environment interactions,



Asthma is made up of different endotypes, each characterized by its unique pathophysiology and environmental and genetic associates. (From Lotvall J, Akdis CA, Bacharier LB, et al. Asthma endotypes: A new approach to classification of disease entities within the asthma syndrome. *J Allergy Clin Immunol* 2011;127(2): 355-60. *Used with permission*.)

where environmental exposure (in this case, drug administration) occurs at a precise dose and time. We will use the controversy on the safety of regular use of beta-2 agonists as an example of this concept in asthma. Concerns about the safety of beta-2 agonists were raised after reports of a sharp increase in asthma mortality during the 1960's and 1970's in countries where potent, non-selective, short-acting beta-2 agonists (SABA) were widely used.[40] However, a large study which compared "regular" to "as-needed" use of albuterol in adults with asthma concluded that regular use and as needed use were equally safe.[41] At the time, several genotype-specific differences in the response to SABA were reported.[42,43] Post-hoc genotyping of the study participants suggested that patients with Arg16Arg genotype in beta-2 adrenergic receptor gene had poorer lung function when using regular albuterol compared to other genotypes, or compared to patients with the same genotype who used albuterol only as-needed.[44] This indicated that the mean group responses in double-blind, randomized, placebo controlled trials may conceal subgroups with poor outcomes. In a subsequent study of asthmatics with Arg16Arg genotype, patients who were not receiving inhaled steroids deteriorated when using regular albuterol and improved when the drug was withdrawn.[45]

It has been reported that the use of long acting beta-2 agonists (LABA) increases the risk of asthma death, particularly in African Americans,[46] and the question arose whether poorer outcomes observed in patients with Arg16Arg genotype using regular SABA may occur with LABAs. To address this, participants in pharma-sponsored clinical trials of LABAs have been retrospec-

tively genotyped,[47,48] and re-analysis suggested no genotype-specific differential response. However, the weakness of these findings may be that the inclusion criterion for most such studies was demonstrable bronchodilator reversibility, making it difficult to extrapolate the findings to many asthma patients who do not meet this criterion. Of note, one study which did not pre-select patients based on bronchodilator reversibility also suggested no genotype-specific response.[49]

Lessons for the development of new treatments

Inconsistencies in genetic studies and studies on the role of environment in asthma may reflect the fact that the relationship between genotype and phenotype in complex diseases is not linear/unidirectional, but modulated by environmental factors.[28] In genetic association studies of asthma, relevant environmental exposures need to be taken into account, and when assessing the effect of environment, we need to factor in genetic predisposition. If we extrapolate this to identification of the new therapeutic targets for asthma, the data presented above suggest that only a proportion of the population will benefit from a specific drug, while the same drug among individuals with different genetic predisposition may cause harm. Thus, it is essential to abandon the concept "one size fits all" in favor of individualized treatments, applicable to individuals with specific susceptibilities. We propose that the way forward is to re-visit the basis of our understanding of the mechanisms of asthma through re-definition of asthma endotypes. These novel endotypes can be discovered using new analytical tools,[15] and will better reflect the underlying molecular pathways, be more relevant to genetic and pharmaco-genetic studies and will allow identification of new targets, treatments and drug molecules.

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Adnan Custovic is Professor of Allergy and Angela Simpson is Professor of Respiratory Medicine, University of Manchester, 2nd Floor, Education and Research Centre, University Hospital of South Manchester, Manchester M23 9LT, UK, Tel: +44 1612 915869, adnan.custovic@manchester.ac.uk.