REVIEW

Gene–environment interactions in asthma

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The underlying pathogenesis of asthma, one of the most common chronic diseases of childhood, is not fully understood. There is a well-documented heritable component to this disease and environmental factors associated with a Westernised lifestyle have also been implicated; recent studies suggest gene–environment interactions are important in the development of this disease. In the absence of a previous review in children, the present report presents the accumulating evidence for gene–environment interactions in asthma pathogenesis. Studies of these interactions in different populations have yielded both expected and unexpected results. This is a new and rapidly developing field where there are currently many more questions than answers.

Asthma is an important and common condition, with one study from the UK reporting that 24% of children had been diagnosed with asthma by 11 years of age.[1] Asthma affects children in many ways and can result in a significantly decreased quality of life, with reduced exercise tolerance and increased school absences.[2] Furthermore the symptoms of asthma diagnosed in childhood persist into adulthood. For example, 50% of children with asthma referred to one hospital clinic had ongoing symptoms in adulthood some 30 years later.[3] As current asthma treatment palliates but does not cure symptoms, better understanding of asthma pathogenesis in children is essential for future advances in asthma management.

Despite asthma's high prevalence and considerable quality of life implications, its pathogenesis in children is not completely understood. What has been established is that asthma is a complex condition, where both genetic and environmental factors are important. Genetic factors are thought to contribute 40–60% of overall asthma risk and genes associated with asthma (''candidate genes'') have been identified on most chromosomes.[4] Genome-wide screens, where areas of greater genetic diversity are identified in asthmatic compared to non-asthmatic groups, suggest that rather than one gene being mostly responsible for asthma, there are approximately 10 genes, each making a moderate contribution to asthma pathogenesis.[4] To further complicate the story, genes that predispose to asthma are not consistent between populations. Most studies that report associations between asthma and genetic factors have examined associations with single nucleotide polymorphisms (SNPs) which occur when a single nucleotide residue is substituted for another. A SNP can be described by one of two methods: the first indicates the position and the substitution (for example, CD14 C-159T where cytosine is substituted with thiamine at the -159 position) and the second is a unique identifier called an ''RefSNP'' or rs number. Many SNPs do not alter the functionality of the protein they code for; however, an SNP that influences the structure of a protein may alter its properties, for example, affinity for receptor binding, and this may confer risk for, or protection against, asthma (examples of these will be described later). Genetic factors are clearly important in asthma but cannot account for the asthma ''epidemic''[5] witnessed within one generation over the latter stages of the 20th century. A number of environmental factors have been associated with asthma, including exposure to house dust mite,[6] an excessively clean environment,[7] tobacco smoke[8] and diet.[9]

In practice, asthma is likely to be caused by combinations of several genetic and environmental factors, all of which should be considered when studying asthma pathogenesis. One recent editorial has stated that ''lumping together groups of individuals faced with different environmental pressures is likely to drastically dilute the recognizable role of genetic determinants to the point of erasing them''.[10] One example of such ''dilution'' would be where a small proportion of the population is genetically susceptible and there is a strong association between exposure and outcome; conventional studies may not be able to detect such an important association as it would most likely be subsumed by the large non-susceptible proportion of the population. In instances where an environmental exposure has an overwhelming influence on asthma pathogenesis, gene–environment interactions are unlikely to be particularly relevant.

Interactions between different genes and different environmental factors could explain the heterogeneity of asthma, which is particularly evident in children. Knowledge of how genes increase susceptibility to certain environmental factors may be crucial to understanding asthma causation and heterogeneity and ultimately lead to the development of novel management strategies and even disease prevention.

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Abbreviations: ETS, exhaled tobacco smoke; GST, glutathione S-transferase; LPS, lipopolysaccharide; NQO1, NAD(P)H:quinone oxireductase; ROS, reactive oxygen species; SNP, single nucleotide polymorphism; TLR, Toll-like receptor

CHALLENGES IN STUDYING GENE–ENVIRONMENT INTERACTIONS IN CHILDREN

The study of gene–environment interactions in childhood asthma is only beginning and extending the pioneering work to date is likely to be challenging in a number of aspects. The methods for analysing DNA and the results are well-established but some important methodological questions remain:

(a) Which asthma outcome should we use? Most studies of gene–environment interactions use doctor-diagnosed asthma as their primary outcome, yet asthma is a heterogeneous condition, particularly in children and especially in preschool children; one doctor's criteria for an asthma diagnosis may not be consistent with those of colleagues. More detailed and objective assessments of study participants are required to phenotype patients with asthma. Methods for objective assessment are already established in older children and include spirometry, skin prick testing and exhaled nitric oxide, but some of these techniques require development for use in younger patients.

(b) How many children should we study? There is an inevitable tension between the cost of accurately phenotyping study participants and their environmental exposures whilst still including sufficient individuals to test a hypothesis. Although more detailed assessments of children cost more in terms of money and time, this expenditure may be offset by the need for fewer participants compared with larger studies where phenotyping is less rigorous; bigger may not necessarily be better![10]

(c) How can we measure environmental exposures? Methods for measuring environmental exposures in children, often considered too difficult in the past, have recently been developed. Validated methodologies for measuring children's exposure to factors present in the diet and indoor and outdoor air are becoming available and will be invaluable in the study of gene–environment interactions.

The first studies describing gene–environment interactions in childhood asthma began to appear in the literature after 2000 and reported associated phenotypic markers such as atopy, bronchial hyperresponsiveness and reduced lung function. In light of the clinical and epidemiological importance of childhood asthma and the potential benefits of further research into its aetiology, we have identified and reviewed the current literature describing the sometimes complex associations between genetic susceptibility, environmental exposures and childhood asthma. Although many gene–environmental interactions are likely to be important in childhood asthma, those which have been most extensively researched can be grouped into those associated with oxidative stress and interactions in which genes influence response to microbial organisms.

GENETICS AND OXIDATIVE STRESS

Oxygen is essential to our survival, yet, paradoxically, we are under constant threat of damage from oxidative stress, which is an imbalance between reactive oxygen species (ROS) and protective antioxidants.[11] Sources of ROS can be considered as either exogenous or endogenous and the respiratory system is constantly exposed to both. Exogenous ROS can be derived from detoxification of harmful exogenous compounds (or xenobiotics) such as diesel exhaust or environmental tobacco smoke.[12] Endogenous ROS can be generated by inflammatory cells during inflammatory and/or infective processes.[12] Left unchecked, ROS damage the airway epithelium and trigger inflammatory cell infiltration, mucous secretion and airway smooth muscle reactivity and proliferation,[12] leading to airway obstruction and hyperresponsiveness, all hallmark features of asthma. ROS can usually be rendered harmless by antioxidant scavengers such as vitamin C[11] and through enzyme-catalysed detoxification processes.[13] Oxidative stress can only occur when ROS production is excessive or antioxidant mechanisms are inadequate.

One study has confirmed an association with an imbalance between oxidative stress and antioxidant mechanisms in asthmatic children.[14] This Turkish study reported that asthmatic children had evidence of increased oxidative status (increased plasma malondialdehyde) and reduced antioxidant status (reduced plasma glutathione) in comparison with non-asthmatic children.[14] Fuller descriptions of antioxidant mechanisms in the context of asthma are given elsewhere.[11 12]

SNPs of genes encoding detoxification enzymes influence the functionality of these enzymes in the lungs and other organs, and this can confer genetic susceptibility to oxidative stress and asthma. These genes include the glutathione S-transferase (GST) superfamily, which encode enzymes grouped into alpha, mu, pi and theta classes (GSTA, GSTM, GSTP and GSTT respectively) and subclasses, for example Mu 1–6.[14] Polymorphisms of GSTP1, GSTM1 and GSTT1 have all been implicated in asthma aetiology through their effect on oxidative stress.

GSTP1

In the lungs, GSTP1 accounts for more than 90% of GST superfamily activity. The function of this enzyme may be altered in association with the Ile105Val SNP (rs947894). The frequency of the Ile105 allele, associated with reduced GSTP1 activity, was found to be 78% in one study of children in Taiwan.[15] The high prevalence of the Ile105Val polymorphism and the importance of GSTP1 to the antioxidant properties of the respiratory tract suggest that the polymorphism is associated with asthma in instances of excessive oxidative stress, and this has been demonstrated. In a cross-sectional study, homozygous genotype Ile105 was associated with increased risk of asthma, but only for children living in highly polluted areas.[15] It remains to be demonstrated whether the proportion of asthmatic children changes when genetically-susceptible individuals move from areas of low to high environmental pollution. In contrast with the Taiwanese study, the previously mentioned Turkish study[14] found no difference between the proportion of asthmatic and non-asthmatic children with homozygous genotype Ile105 (55% vs 51%, respectively). Somewhat unexpectedly, this group found that the 22 asthmatic children homozygous for Val105 were at increased risk for severe asthma compared with asthmatic children with other Ile105Val genotypes; this observation clearly needs to be confirmed in a larger population. Apparently contradictory findings for the relationship between genes and asthma in different populations are common; different levels of environmental exposures may be important and this will be discussed later.

The GSTP1 Ile105 allele in mothers, but not fathers, has been found to be associated with increased atopy and bronchial hyperresponsiveness in children.[16] In this paper, the authors suggest that increased ROS in genetically susceptible mothers cross the placenta and damage the developing fetal lungs.[16]

GSTM1 and GSTT1

Both GSTM1 and GSTT1 have null alleles that have no active gene product, that is no antioxidant enzyme. In a cross-sectional, school-based study in Germany, the $GSTM1^0$ genotype (homozygosity for the null allele) occurred in 51.6% of the population, whilst the frequency of $GSTT1^0$ was 17.8%.[17] The relatively high prevalence of these genotypes and total

absence of gene product suggests that these genes are relevant to the antioxidant properties of the lungs, particularly in situations of high ROS exposure. This was demonstrated in a study that showed that the increased risk of asthma from exposure to environmental tobacco smoke was limited to those with the $GSTM1^0$ or $GSTT1^0$ genotypes.[17]

Dietary factors may be important to asthma pathogenesis, and dietary vitamins can act beneficially as antioxidant scavengers. A recent randomised controlled trial in Mexico showed that, while vitamin C and E supplementation prevented a decline in lung function associated with ozone exposure, only children with $GSTM1^0$ showed a statistically significant benefit.[18] This suggests that vitamins may help to overcome genetic susceptibility to antioxidant stresses, such as in children with $GSTM1^0$, in a situation of increased oxidant load, although a clinical benefit, in terms of improved symptoms, has not yet been demonstrated.

Gene–environmental interactions may also be important before birth. For example, a large cross-sectional questionnaire-based study found that exposure to maternal smoking in utero was associated with increased risk of asthma and bronchial hyperresponsiveness and reduced lung function but only for children with the $GSTM1^0$ genotype.[19] Clearly, mothers who smoke during pregnancy are likely to smoke afterwards and an apparent post-natal effect may in fact represent antenatal exposure, or vice versa.

OTHER GENES ASSOCIATED WITH ANTIOXIDANT SUSCEPTIBILITY

Some genes modulate the interactions between GST genes and environmental oxidant exposures. One example is the gene coding for the detoxification enzyme NAD(P)H:quinone oxireductase (NQO1). In addition to its detoxification properties, NQO1 also catalyses the activation of quinones to hydroquinones that may react to form ROS.[20] Thus NQO1 reduces oxidative stress but may paradoxically also produce ROS. Therefore, patients with the inactive Ser allele of the NQO1 Pro187Ser SNP (rs1800566) should theoretically be less likely to suffer the effects of oxidative stress and this interaction was explored in a case–parent triad design study.[21] A complex interrelationship was observed where children with the $GSTM1^0$ genotype and at least one 187Ser allele had a significantly reduced risk of asthma compared with those with no Ser alleles. Furthermore, the protective effect of the Ser allele within the $GSTM1^0$ group was limited to children with non-smoking parents.[21] Effectively, the 187Ser allele seems to partly protect against increased oxidative stress conferred by the $GSTM1^0$ genotype, but this protection is overwhelmed by environmental tobacco smoke. This study gives insight into how gene–gene interactions may influence gene–environment interactions.

GENETICS AND ENCOUNTERS WITH BACTERIAL INFECTION

Early encounters with microbial antigens are thought to be critical to the later development of allergy and, by association, asthma. The hygiene hypothesis proposed that reduced encounters with bacteria in early life will be associated with increased allergic conditions in later life and was developed by researchers who noted an apparent protective influence of older siblings on the development of hay fever.[7] Several genetic polymorphisms related to our capacity to interact with bacteria have been studied in the context of childhood asthma and the hygiene hypothesis. A brief summary of the interactions between the human immune system and bacteria and how these may have life-long implications is given below.

At birth, the immune system is vulnerable to becoming pro-allergic and initial encounters with bacteria are thought to determine whether the developing immune system becomes biased towards or away from allergy.[22] These initial encounters involve Toll-like receptors (TLR), of which there are four types, which are expressed on the outer membranes of cells important to the immune system. The TLR-4 is the specific receptor for the most potent and important bacterial antigen, lipopolysaccharide (LPS).[23] Binding of LPS to TLR-4 is dependent on another molecule (a ''signalling partner'') named CD14. CD14 is a molecule expressed on the outer cell membrane of cells in the innate immune system, for example, monocytes and neutrophils. CD14 is also found free in the plasma where it can suppress the activity of cells important to the adaptive immune system, and in particular T lymphocytes. Given that initial encounters between microbial antigens (including LPS) and the innate and adaptive immune system appear to be critical in the development of allergic sensitisation, genetic variations that alter the ability of TLRs and CD14 to interact with LPS may be important to the development of allergy and asthma.

Toll-like receptors

Epidemiological studies have shown that children in farming households and children exposed to stables or farm milk in utero or during the first year of life have a decreased risk of atopy. These associations are thought to be driven by high exposures to LPS in early life.[24] Associations between atopy and LPS become clearer when considering SNPs in genes coding for TLR-2 and TLR-4 that confer enhanced binding to LPS. In a study of school children in Austria and Germany, the T allele of the TLR-2 promoter polymorphism A-16934T (rs4696480) was found to protect against atopic asthma and hay fever.[25] However, this association was limited to children in farming households. The risk of asthma in non-farming households was unaffected by the TLR-2 genotype. It is possible that the T allele of the TLR-2 results in increased TLR-2 expression, allowing the immune system to recognise and respond to endotoxin more efficiently.[25] However, a certain concentration of endotoxin is required for this polymorphism to be beneficial.

Eder *et al*[25] found a statistically significant reduced risk of atopy in children with a missense polymorphism at position +4434 of TLR-4 (rs10759932) who were also exposed to high levels of endotoxin. They also found a non-significant trend suggesting the same polymorphism increased the risk of atopy in patients with low levels of exposure.[25] This non-significant trend may have been due to random error or may indicate further complexity of this gene–environmental interaction. For instance, there may be an interaction between SNPs in genes coding for TLR and CD14.

CD14

The C allele of the CD14 promoter SNP C-159T (rs2569190) is associated with increased circulating CD14[26] and the C-159T polymorphism has been associated with altered risk for allergy and asthma in several adult and paediatric populations. Although most studies find the T allele confers apparent reduced risk,[26] some find the same allele confers increased risk,[27] whilst yet others find no association between the T allele and atopy.[28] These apparently inconsistent results may simply reflect random findings in underpowered studies. However, an alternative explanation is that these findings represent a consistent but complex gene–environment interaction. One author has proposed the endotoxin switch theory,[23] where the C allele confers risk at low exposures of LPS whilst the T allele confers risk at high exposures of LPS, and this may account for apparent inconsistencies between studies.

At least two studies of SNPs in the gene coding for CD14 support the endotoxin switch theory. A study of German

children found the C allele of the CD 14/-260 SNP (rs2569190) to be associated with increased IgE for those exposed to domestic animals (lower LPS exposure), whilst children carrying the C allele exposed to farm animals (higher LPS exposure) had reduced IgE.[29] The investigators involved in the Manchester Asthma and Allergy Study sought to relate atopy to the C-159T SNP in the context of LPS exposure.[30] As hypothesised, the Manchester team found that CC homozygotes had reduced risk of atopy at high levels of endotoxin exposure but increased risk of atopy at lower endotoxin levels in comparison with children carrying at least one T allele. Additionally, for CC homozygotes, high levels of endotoxin exposure (protective against atopy) increased the risk of non-atopic asthma, while other genotypic groups did not show this association.[30] This latter association suggests that CD14 SNPs might confer asthma risk independent of atopy, for example by influencing lung function.

Choudhry *et al*[31] investigated the association between exposure to exhaled tobacco smoke (ETS, which contains endotoxin), CD14 polymorphisms and asthma phenotype in Mexican and Puerto Rican adults and children. Those individuals homozygous for the T allele of the C-159T polymorphism who were also exposed to ETS had the lowest IgE values and those who carried at least one G allele of the G+1437C SNP had an 8% reduction in lung function but only when exposed to ETS.[31] The results of this study suggest that, like the Manchester study, CD14 SNPs may be important to asthma pathogenesis via mechanisms that are dependent and independent of atopy.

CONCLUSION

Gene–environmental interactions for childhood asthma are complex. There are a vast number of possible combinations of genetic and environmental factors, and different combinations may confer different asthma risks and phenotypes. It is likely that many important gene–environment interactions are not yet described. With further hypothesis-driven research, knowledge of these interactions is likely to develop understanding of asthma aetiology and may aid treatment and prevention.

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