GENE-ENVIRONMENT INTERACTION IN CHILDHOOD ASTHMA

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The importance of early life environmental influences on the etiology of asthma is implied by the observed geographic and temporal variation in the prevalence of the disease among children. There is evidence pointing to the role of exposure to allergen, various aspects of diet and hygiene-related factors in the etiology of asthma. There is also evidence that heritable factors influence the impact of hygiene-related exposures on the risk of having asthma. A number of important gene-environment interactions have been identified. These interactions point to the biology of environmental exposures as the involved genetic variation is suggestive of certain underlying mechanisms. Polymorphisms within genes coding for the toll-like receptor-lipopolysaccharide (TLR-LPS) signaling pathway may underlie variations in effects of hygiene-related exposures, including specifically endotoxin, on the risk of developing allergic sensitization and allergic disease. This review presents recent findings illustrating the role of gene-environment interactions in childhood asthma susceptibility.

Asthma is an important and common condition: a UK study reported 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 asthma diagnosed in childhood persists into adulthood. Despite asthma's high prevalence and considerable quality 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. Many studies have shown that there is a genetic accumulation in the development of asthma and allergic disorders. Genetic factors are thought to contribute 40-60% of overall asthma risk and genes associated with asthma ("candidate genes") have been identified on most chromosome (3). Interactions between different genes and different environmental factors could explain the heterogeneity of asthma, which is particularly evident in children.

In the past decades, more than 200 asthma candidate genes have been identified using genetic association studies, positional cloning and knockout mouse approaches (4). In the recent years it has been possible to perform whole-genome investigations large due to the genome-wide association studies (GWAS) (3;5-7), that have soon shown to be powerful tool to identify novel loci and susceptibility variants for common diseases.

In the light of the clinical and epidemiological importance of childhood asthma and the potential benefits of further research into its etiology, we have review the current literature describing the sometimes complex associations between genetic susceptibility, environmental exposure and childhood asthma.

GENETICS AND ENCOUNTERS WITH BACTERIAL INFECTION.

Several studies showed that heritable factors influence the impact of hygiene-related exposures on the risk of having asthma. The term "hygiene hypothesis" was attributed to David Strachan, who coined in 1989 to explain his observation that hay fever was less common in children who grew in large families (8). Since then, a considerable body of epidemiological evidence has accumulated around the protective effect on allergy

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development exerted by environmental and lifestyle factors that seem to have a link with hygiene. Further observations showed that exposure to other children reduces the risk of being allergic and, consequently, of having allergic illnesses such as asthma and hay fever (9-11).

In the absence of a large family, a similar protective effect was found subsequently to exposure to children in early child care (12, 13).

Childhood exposure to animals also reduced the risk of acquiring allergic disease. This exposure may occurs in farm (14, 15) or in the domestic environment (16-18). The "hygiene hypothesis" suggests that the resulting changed and reduced pattern of exposure to microorganisms has led to disordered regulation of the immune system, and hence to increase in certain allergic and inflammatory disorders. A reduced encounters with bacteria in early life will be associated with increased allergic conditions in later life. Several genetic polymorphisms related to the capacity to interact with bacteria have been studied in the contest of childhood asthma and the hygiene hypothesis.

Toll-like receptors

At birth, the immune system is vulnerable to become pro-allergic and initial encounters with bacteria are thought to determine whether the developing immune system becomes biased towards or away from allergy (19). These initial encounters involve Toll-like receptors (TLRs).

The Toll protein was originally identified in *Drosophila melanogaster* as a protein important for embryogenesis and innate immune response. Currently, 11 different Toll-like receptors are known in humans. They are transmembrane receptors containing two important structural domains: the Toll/IL-1 receptor (TIR) domain, which is also a common structure in members of the interleukin (IL)-1 receptor family, and the leucin-rich repeat (LRR) domain. The TIR domain conveys intracellular signaling, whereas the extracellular LRR domain is involved in ligand recognition. TLRs bind phylogenetically conserved microbial structures, the so-called pathogen-associated molecular patterns (PAMPS). TLRs are involved in the first immune response to both acute infections and noninvasive microbial products. TLR4, for example, represents the receptor for endotoxin (lipopolysaccharide, LPS), a part of the cell wall of Gram-negative bacteria that is found ubiquitously in the environment. Endotoxin has been suggested as one of the major factors mediating the protection from allergic disease (20).

Associations between atopy and LPS become clearer when considering SNPs (single nucleotide polymorphisms) in genes coding for TLR -2 and TLR-4 that confer enhanced binding to LPS (21).

TLR2 receptor form dimers with TLR1 or TLR6. The TLR1/TLR2 dimer recognizes triacylated lipopeptides, while TLR2/TLR6 dimer recognizes diacylated lipopeptides. TLR2 responds to a wide variety of microbes, such as Gram-positive bacteria, Gram-negative bacteria, mycobacteria, mycoplasma (22), that are capable to induce severe pulmonary infections. Recent studies showed that the TLR2 receptor have an important role in the immune-modulate disease pathogenesis (22,23)

In a study of school children in Austria and Germany, the T allele of TLR-2 promoter polymorphism A-16934T (rs4696480) was found to protect against atopic asthma and hay fever (24). However, this association was limited to children in farming household. The risk of asthma in non-farming households was unaffected by the TLR-2 genotype. Probably, the T allele of the TLR-2 results in increased TLR-2 expression, allowing the immune system to recognize and respond to endotoxin more efficiently (24).

In a recent study, Kormann et al (25) showed a significant association between genetic variants in TLR receptors 1 and 6, forming complexes with TLR2, and atopic asthma in large groups of European children. These data suggested that the TLR2 pathway with the associated components TLR1 and TLR6 are factors in the innate immune system that contribute to the genesis of asthma and allergy. It is possible that the signals by the TLR2 network trigger effects by the adaptive immune system, resulting in elevated total and specific IgE levels, asthma, and atopic diseases (26).

TLR4 is essential for responses to LPS, a glycolipid specific to Gram-negative bacterial cell walls (27). Of note, ligand-dependent cell activation through TLR4 and TLR2 (and possibly other TLRs) requires additional molecules, first and foremost CD14, which is expressed both as a GPI-linked and a soluble protein (28,29). CD14 is the receptor that binds LPS and transfers it to TLR4, thus forming the CD14-TLR4 complex. Targeted disruption of the TLR4 gene resulted in abrogation of the responses to LPS (30). In humans, common mutations in the TLR4 gene are associated with differences in LPS responsiveness (31).

Two coding variations were discovered in the TLR4 gene (Genbank accession no. NM 138554), Asp299Gly and Thr399Ile SNPs, that were associated with hyporesponsiveness to inhaled endotoxin in humans (32). Fageras et al showed a direct association of the TLR4 Asp299Gly polymorphism with asthma in Swedish school children (33). Saçkesen et al (34) also found that the heterozygosity for the same SNP was associated with mild forms of asthma in Turkish children, whereas three other studies showed no differences in the overall risk for asthma between carriers of the wild-type and the

less frequent genotype (35, 37). On the other hand, the Asp299Gly polymorphism was associated with a modified response to endotoxin (37), indicating gene-environment interaction.

Recently, Penders et al (38) studied within the KOALA birth cohort study (Child, Parent, Health, Focus on Lifestyle and Predisposition) a gene-environment interaction among gut microbiota, genetic variation, and the development of atopy. They showed that the E. coli colonization was associated with a decreased risk of sensitization in children with the TLR4 rs10759932 TT genotype, but not in children with the C allele.

Moreover, it has been found a statistically significant reduced risk of atopy in children with this polymorphism and who were exposed to high levels of endotoxin (24).

CD14

CD14 play a prominent role among gene-environment interaction studies of asthma-related phenotype and it has proven a rewarding gene candidate. It is a glycosylphos phatidylinositol (GPI)-linked protein containing LRRs, which is involved, together with an LPS-binding protein (LBO), in response to LPS. CD14 is expressed on the surface of macrophages and monocytes and is also present in soluble form. It binds LBP and delivers LPS-LBP to the TLR4-MD-2 complex.

CD14 may be considered a crucial link between non-adaptive and adaptive immune responses to environmental antigens. A recent study compared associations between allergic diseases and CD14 -159C/T in Finnish and Russian Karelian women. Finnish Karelian females had a higher prevalence of allergic disease than Russian Karelian ones, yet both populations belong to the same ethnic group. The CD14 -159 risk allele for atopic phenotypes in Finnish Karelia turned out to be the protective allele in Russian Karelia. Indeed, the risk allele was C in Russians and T in Finns. Thus, an Eastern or Western environment appeared to affect risk of allergic disease in adult women through opposite alleles (39). Moreover, the C allele of the CD14 promoter SNP C -159T (rs2569190) was associated with increased circulating CD14 (40) and the C-159T polymorphism has been associated with altered risk for allergy and asthma in several adult and pediatric populations (41-43). The T allele of the CD14 -159 (C/T) promoter polymorphism was associated with a decreased total serum IgE in a cohort of children from Tucson (Arizon) (40), whereas no association of this SNP with allergy or serum IgE levels was evident in a large German cohort. In the Huttrites, an isolated population from South Dakota, the -159T allele was instead associated with an increased risk for atopy (44). Thus, although most studies found the T allele conferred apparent reduced risk (40), some found the same allele conferred increased risk (45), whilst yet others found no association between the T allele and atopy (46). 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 theory" (44), 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. This hypothesis may be supported by the observation that this CD14 polymorphism leading to a C>T nucleotide substitution, located 260 bp from translation start site and 159 bp from transcription start site, alters CD14 promoter activity *in vitro* by decreasing the affinity of Sp protein binding and thus enhancing transcriptional activity (47). Thus, it has been hypothesized that the level of endotoxin exposure may influence the switch over from the Th2-biased cytokine profile at birth to a Th1-biased cytokine profile in early childhood, and that the endotoxin levels might interact with the CD14 genotype to confer either risk to or protection from atopic phenotypes later in life (44).

Significant gene-environment interactions between variation in CD14 and TLR genes and country living during childhood were found for 10 SNPs (48). In skin test-positive subjects carrying CD14/-260 CC, country living protected against asthma, whereas country living was not associated with asthma in subjects who were atopic and carried CD14/-260T. Therefore, TLR2 and CD14 SNPs were associated with asthma and atopic asthma respectively. Moreover, SNPs in CD14 as well as TLR2 and TLR4 modified associations between country living and asthma (49).

Recently, 3,062 children were selected from three birth survey cohorts: the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) study, the Prevention of Athma in Children (PREVASC) study, and the KOALA (?) study. They were genotyped for polymorphisms of CD14 gene. Moreover, they were tested for association with serum total and specific IgE and interaction with tobacco smoke and pet exposure at 1, 2, 4 and 8 year ages. In CD14, the rs2569190 TT (CD14-260C/T) and rs2569191 CC genotypes were associated with lower IgE and decreased risk of sensitization at 4 and 8 yrs in children exposed to pets, with an opposite effect in non-exposed children. These results were found in separate cohorts. This study shown that atopy is significantly influenced by CD14 in interaction with exposure at 4 and 8 years (50).

To study the interaction between CD14 and TLR4 genes and gut microbiota, fecal samples of 957 one-month-old infants from KOALA study, were collected and quantitatively screened for E. coli. Fourteen

haplotype-tagging polymorphisms in TLR4 and CD14 were genotyped in 681 children. All children suffered from atopic diseases. Most SNPs showed no significant interaction with E.coli exposure for eczema and allergic sensitization. However, a slightly significant interaction was found between the CD14-159C/T SNP and E.coli in children with allergic sensitization.

Genome-wide association studies.

Although several genetic studies have been performed to understand the pathogenesis of the childhood asthma, the role of many genes has not been fully elucidated still.

Recently, genome-wide associations studies (GWSA) are expected to lie in their ability to discover truly novel disease candidate genes, especially those associated with moderate risks (4).

In an interesting genome-wide association study for asthma, more than 317,000 SNPs were characterized in DNA from 994 patients with childhood asthma and 1243 subjects without asthma by using family and case-control panels. Multiple markers on chromosome 17q21.1 were found to be strongly associated with childhood asthma (51). The association was independently replicated in 2320 subjects from a cohort of German children and in 3301 subjects from the British 1958 birth cohort (51).

Ricci et al (3) performed a pooled GWSA and individual genotyping in 269 European children with allergic respiratory diseases comparing allergic children with and without asthma. This study showed that the most significant SNP was located inside the coding sequence of C5, that was already identified as an asthma susceptibility gene (52). Moreover, it has been shown that the other studied loci have an essential role in the regulation of the bronchial physiopathology, as immune- or inflammation-mediated mechanism and airway smooth muscle contraction.

CONCLUSIONS

Gene-environmental interactions for childhood asthma are complex. There is a large number of possible combinations of genetic and environmental factors, and different combinations of genetic and environmental factors may confer different risk and phenotypes. Asthma is likely a syndrome rather than a single disease entity, in which different pathways eventually result in various phenotypes of variable airway obstruction. The identification of novel genes for asthma may suggest that many genes with small effects rather than a few genes with strong effects contribute to the development of asthma. These genetic effects may in part differ with respect to a subject's environmental exposures, although some genes may also exert their effect independently of the environment.

Therefore, an important goal for future studies is to elucidate the complex interaction between genes and environment in this disease. Identifying the most important asthma genes in the context of environmental factors could facilitate the setting up of a genetic risk profile for the development of asthma. This would enable us for the first time to take preventive measures early in live for children with an increased genetic risk to develop allergic diseases.

There are important comprehensive approaches on how genetic factors influence interaction with the environment in the childhood asthma, for example GWAS and linkage analysis. Although GWAS have the power to identify mainly common variants and explain a small proportion of hereditability of childhood asthma, these studies may contribute to our understanding of gene-environment interactions and of their impact on complex disease susceptibility. On the other hand, linkage analysis can detect different types of genetic factors within one locus or several loci, including rare variants segregating in families.

Knowing the most important asthma genes would also help in the design of new drugs which are more specific, effective and save. Thus in consideration of the new technologies and possibilities in asthma genetics research the expectations for the years to come are high.

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