Gene–Environment Interactions Associated with the Severity of Acute Asthma Exacerbation in Children

Considerable progress has been made in understanding the environmental and genetic factors that contribute to the inception of asthma early in life. However, the factors that regulate the actual severity of acute exacerbation or the frequency with which severe exacerbations occur in children are likely distinct from those that contribute either to disease inception or baseline disease phenotype (e.g., References 1, 2). Investigating the basis of variation in the clinical phenotype of rhinovirus infection represents an opportunity to understand the biological mechanisms that predispose some children to severe asthma exacerbation. Rhinovirus is a dominant cause of morbidity among children with asthma, and rhinovirus-triggered asthma exacerbations are more severe than exacerbations caused by allergic triggers (3). However, there is still marked interindividual variation in the susceptibility to and severity of rhinovirus-triggered asthma exacerbation, ranging from mild symptoms to life-threatening episodes (3–5).

Several lines of evidence suggest that the severity of rhinovirus-triggered asthma exacerbation is influenced by gene–environment interactions between rhinovirus and host genetic background (Figure 1A) (6–8). A fascinating story has taken shape recently that clarifies one mechanism by which this might operate, and illustrates how genetic association studies can identify novel disease mechanisms. Beginning with a genome-wide association study (GWAS), a novel nonsynonymous coding SNP was identified at the cadherin-related family member 3 (*CDHR3*) locus, which strongly associated with recurrent episodes of severe asthma exacerbations during childhood (9). The cumulative risk of asthma hospitalizations among those homozygous for the risk allele at SNP rs6967330 was about twofold higher compared with those homozygous for the wild-type allele. *CDHR3* is a transmembrane protein belonging to the cadherin protein family, the function of which was unknown at the time of the discovery GWAS. However, evidence is now pointing to *CDHR3* playing a pivotal role in mediating the effects of certain rhinoviruses, specifically rhinovirus C.

Initially, after an *in silico* genome-wide screen to identify putative rhinovirus C receptors, Bochkov and colleagues (10) found that transfecting *CDHR3* into cultured cells confers the ability to support rhinovirus C binding and viral replication. Mutating cysteine to tyrosine at position 529 of the *CDHR3* protein, which mimics the effect of the rs6967330 risk allele, substantially enhances *CDHR3* cell surface expression and rhinovirus C binding and replication compared with the wild-type protein. Collectively,

these results point to *CDHR3* as a functional rhinovirus receptor *in vitro*. If the same relationship exists *in vivo*, is it possible that increased susceptibility to rhinovirus C infection resulting from the *CDHR3* risk allele is the causal mechanism underlying the increased risk of severe asthma exacerbation?

The answer to this question comes into better focus as a result of the study presented in this issue of the *Journal* by Bønnelykke and colleagues (pp. 589–594) (11). The authors found that the rs6967330 risk allele was reproducibly associated with the detection of rhinovirus C in the nasal aspirates of young children with moderate to severe respiratory infections, but not with the detection of other viruses, including other rhinovirus species. These effects were additive, meaning that those heterozygous for the risk allele had about a 50% increased risk of rhinovirus C infection, whereas those homozygous for the risk allele had a nearly twofold increased risk of rhinovirus C infection. These results nicely parallel the original *CDHR3* GWAS study, which showed a similar additive effect for the risk of severe asthma exacerbation (9). The observation that the *CDHR3* risk allele is associated with both the frequency and severity of rhinovirus C respiratory illness suggests that variation at the *CDHR3* allele confers an increased risk of severe asthma exacerbation through an interaction with rhinovirus C: the risk allele increases cell surface expression of *CDHR3*, resulting in increased rhinovirus C binding and replication, ultimately increasing the risk of acute asthma exacerbation (Figure 1B). The plausibility of this model is further substantiated, because rhinovirus C is associated with increased frequency and severity of asthma exacerbation in children compared with other respiratory viruses (12).

The gene–environment interaction between *CDHR3* and rhinovirus C sheds light on one mechanism that might regulate acute asthma exacerbation severity. There is likely much more to learn: variation at the *CDHR3* allele explains only a small fraction of the risk of severe acute asthma exacerbation, and yet a substantial share of the interindividual variation in acute asthma exacerbation severity is likely attributable to host genetic background (13–15). In addition, although *CDHR3* and rhinovirus C appear to interact selectively, rhinovirus A also contributes substantially to the burden of asthma exacerbation. Are there additional genetic variants that selectively regulate the response to other viruses that trigger asthma exacerbation?

To date, there have been few studies that have sought to systematically identify genetic variants that contribute to interindividual differences in the frequency and severity of rhinovirus-triggered asthma exacerbation (7, 8). This is partly because GWASs are poorly powered to investigate gene–environment interactions. One approach to circumvent this limitation is to focus on extreme phenotypes (16). Another approach is to investigate gene expression phenotypes, which vary

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Figure 1. Model of gene–environment interactions. (A) Acute asthma severity is increased when the susceptible *CDHR3* genotype occurs together with a triggering environmental exposure, namely rhinovirus infection. Those homozygous for the *CDHR3* risk allele (rs6967330-A) are about twice as likely to be hospitalized for asthma exacerbation by age 6 years as those who are homozygous for the wild-type allele (rs6967330-G) (9). Note that the phenotype associated with the *CDHR3* risk allele is expressed only when it occurs together with the triggering environmental exposure. (B) The *CDHR3* risk allele increases cell-surface expression of CDHR3, resulting in increased rhinovirus C binding and replication. Enhanced rhinovirus C virulence triggers a sequence of events leading to increased severity of acute asthma exacerbation. CDHR3 = cadherin-related family member 3.

depending on an individual's genotype, often with large effect sizes. These gene expression phenotypes can therefore be mapped to the genome, allowing identification of genetic variants, or expression quantitative trait loci (eQTLs), that regulate gene expression. It is becoming increasingly evident that genetic regulation of gene expression is likely central to the genetic basis of phenotypic variation (16). Identifying condition-specific eQTLs (i.e., mapping eQTLs in the presence and absence of a specific environmental exposure) is a novel approach to characterizing the global regulatory landscape of gene expression response in a specific environmental context, such as rhinovirus (7).

The evolving story of *CDHR3* and rhinovirus C provides key insights into the possible mechanisms by which gene–environment interactions may influence the severity of acute asthma exacerbation. In addition, these findings also raise the possibility that the *CDHR3* risk allele increases the frequency of viral-inducing wheezing illness during infancy, which, together with atopy, is strongly associated with the inception of asthma later in life (12). Further characterizing the genetic basis of interindividual variation in the clinical phenotype of rhinovirus infection will provide insights into the biological mechanisms that predispose some children to severe episodes of rhinovirus-triggered asthma exacerbation, and will ultimately improve risk stratification and treatment. ■

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Household Air Pollution and Chronic Obstructive Pulmonary Disease “A Riddle, Wrapped in a Mystery, Inside an Enigma”

The World Health Organization (WHO) predicts that chronic obstructive pulmonary disease (COPD) will be the third leading cause of death worldwide by 2030 (1). It also lists household air pollution (HAP) from emissions from cooking and heating with biomass fuels as a major risk factor for COPD. The Global Burden of Disease comparative risk assessments have included COPD due to HAP as a major cause of disability-adjusted life years (years of life lost plus years lost to disability) for more than a decade (2). Both the WHO and the Global Burden of Disease efforts have based their assessment of risk of COPD due to HAP on a relatively large but heterogeneous body of observational epidemiological studies that suggest associations between reported exposure to HAP from domestic cooking with solid fuels (e.g., wood, coal, dried dung, and crop waste) and various COPD outcomes (3–7). Because approximately 40% of the world's households still cook and heat with solid fuels, it is often held that among women in low- and middle-income countries (LMIC) who have low rates of tobacco smoking, HAP is the leading cause of COPD (8).

The epidemiological database on the HAP–COPD association does suffer from a number of limitations, however. First, virtually all of the published studies used self-report of solid fuel use as the exposure variable rather than actual measurement of pollution. Second, there is considerable heterogeneity in outcome variables in these studies, from self-report of symptoms of chronic bronchitis, to self-reported diagnosis, to spirometry. And even when spirometry was performed, it was not done after administration of inhaled bronchodilator. Given the potential importance of HAP as a risk factor for COPD in LMIC, the need for more reliable data on the association is great.

The results of two studies that were conducted to address this need are published in this issue of the *Journal*, by Amaral and colleagues (pp. 595–610) (9) and Siddharthan and colleagues (pp. 611–620) (10). Both studies were large in scale, were conducted

in multiple countries, performed spirometry according to European Respiratory Society/American Thoracic Society standards, used post-bronchodilator data to assess COPD, and did appropriate statistical analyses adjusting for multiple potential confounders. Although it would have been reassuring if the results of these two studies were congruent, the unfortunate reality is they are not.

The meta-analysis of results from 25 BOLD (Burden of Obstructive Lung Disease) study sites by Amaral and colleagues found no association between airflow obstruction and use of solid fuels for cooking or heating, and this was true for both LMIC and high-income sites (9). Chronic phlegm, however, was more likely to be reported among female never-smokers and those who had been exposed to HAP for 20 years or more. A recent study from Canada also reported that among never-smoking women, biomass fuel used for heating was associated with COPD (11).

The pooled analysis of data from 13 studies in LMIC by Siddharthan and colleagues showed strikingly different results (10). Participants with HAP exposure were 41% more likely to have COPD than those without the exposure, and the population-attributable risk of COPD prevalence due to HAP was slightly greater than that due to cigarette smoking (10).

How does one reconcile the conflicting results of these two large-scale, multicountry analyses? First, it must be acknowledged that both studies, although well conducted, are cross-sectional observational studies. Both used population-based, random sampling recruitment methods that make selection bias unlikely. Information bias, however, is a problem for both studies, because self-reported use of solid fuel for cooking and/or heating (9) or biomass as the primary source of fuel in the home (10) is subject to exposure misclassification. Although such exposure misclassification tends to bias toward the null, this is not always the case. A strength of both studies is the presumably high-quality spirometry data collected, although this too can be problematic if symptomatic participants are excluded from data analysis

“A riddle, wrapped in a mystery, inside an enigma”—Winston Churchill.

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