

study did not replicate the original finding. Moreover, a direct comparison of ORs in the discovery and replication sample allows a less biased evaluation of the evidence for replication compared with just evaluating *P* values from association tests that only investigate if the ORs are significantly different from 1.0.

The most difficult issue in replication studies and in the evaluation of power is accurately estimating the effect size. Among the factors that are relevant in the consideration of effect size are: (1) we nearly always attempt replication using proxies of causal variants (i.e., using markers in LD with the causal variants) because in the majority of situations the causal/functional SNP is not known; (2) the quality of the proxy can be sample dependent, especially when samples of different race are used (e.g., replication of a SNP associated with asthma in whites using an African American dataset may fail because the proxy SNP may be in LD with the causal SNP in whites but not in African Americans), (3) functional variation may be less well covered than nonfunctional variation on the high-throughput platforms (3), and (4) the effect sizes in the discovery and replication samples are very similar only when then the two cohorts are similar with respect to phenotype (e.g., adult vs. childhood asthma; atopic vs. nonatopic asthma), relevant environmental variables (e.g., exposure to cigarette smoke or farm animals in infancy), and ascertainment (e.g., family-based ascertainment vs. case-control studies; multiplex vs. simplex cases). In fact, there are many examples that demonstrate the importance of considering “environment” in genetic studies. Replications of associations with the *CD14* or *HLA-G* genes were only realized when the samples were stratified by animal/endotoxin exposure (4–7) or maternal affection status (8, 9), respectively. Many other “failed” replications also likely represent our failure to understand the relevant environmental contexts that influence the genotypic effects at the candidate SNP.

Considered in this context, the Rogers and colleagues study tells us little about the 15 genes that were not replicated. On the other hand, this study provides additional evidence for association with 24 asthma candidate genes and is likely the first of many studies that will use genome-wide SNP genotypes for candidate gene studies. As with this study, it will ultimately be much easier to prove a gene's involvement in asthma patho-

genesis through replication than to prove that an associated SNP in one study is actually a false positive result.

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Causal Links between RSV Infection and Asthma No Clear Answers to an Old Question

“We have shown strong evidence for a causal relationship of winter viruses with early childhood asthma,” concluded Wu and colleagues in a recent issue of this *Journal* (1). “RSV infection does not cause asthma but is an indicator of the genetic predisposition to asthma” counter Thomsen and colleagues (pp. 1091–1097) in this issue (2). The two articles follow up on a discussion that has been going on for decades. It is undisputed that infants hospitalized with a lower respiratory tract infection caused by respiratory syncytial virus (RSV) more often report subsequent childhood wheeze. What remains unclear is the direction of causation: does RSV infection confer a long-term change in the host, which increases the risk of subsequent asthma? Or is a hospitalization with RSV bronchiolitis simply an early marker of an underlying predisposition for reversible airway disease (i.e., asthma)?

What do the two new articles contribute to this debate? Wu and colleagues, analyzing data from 95,000 infants from a state-based health-care program in Tennessee, found that children

aged 4 months at the winter virus peak had an increased risk of bronchiolitis in infancy and of asthma during childhood (1). These results add convincing evidence to another old dispute—whether there is an association between season of birth and risk of asthma (3, 4)—but they contribute little to clarify causation. The figures which suggest that children born in September bear the highest risk of later asthma could be explained, as the authors suggest, by the timing of birth 4 months before the winter virus peak. Other explanations include being born 8 months before the pollen peak or some critically timed exposure during fetal life (5); in short, any seasonally varying exposure. Although the authors point out that the curves shifted with changing virus peaks from year to year, a statistical test supporting this statement, i.e., comparing the infants' age at the winter virus peak to their age at a fixed calendar date, is missing. Moreover, in the 20% of children who had had clinical bronchiolitis, risk of asthma was not predicted by the age they

were ill, but by their age when the national virus peak occurred. This is surprising. If we hypothesize that RSV bronchiolitis confers developmental damage, this should happen when the child is ill but not when other children are ill. Finally, the parallel trend in hospitalizations for bronchiolitis and asthma in the U.S. is prone to ecological fallacy and provides no evidence for an association between RSV infection and asthma at the individual level, much less for causality.

The current article by Thomsen and colleagues makes a more convincing attempt to detect causality in the RSV-asthma association (2). Using a large dataset of 8,280 twin pairs in Denmark, they fitted genetic variance components and direction of causation models to their data. This method draws on cross-trait cross-twin correlations to resolve both the genetic relatedness and the direction of causation between two traits, in this case, RSV hospitalization and asthma (6, 7). The authors found that the association between RSV and asthma was essentially due to genetic effects shared between these traits. Furthermore, a model in which RSV hospitalization causes asthma could be rejected by statistical testing, whereas one in which asthma causes RSV could not. These twin data thus quite clearly contradict the hypothesis of a causal effect of RSV on asthma, suggesting that the observed association is due to shared genetic predisposition.

Both articles suffer from poor phenotype definitions for RSV infections and for asthma, which makes the findings hard to interpret. An RSV infection was simply defined as discharge diagnosis with verified RSV infection. Whereas 70% of infants have experienced an RSV infection by the age of 1 year, and 100% by the age of 2 years, the host response to the virus varies greatly, including upper respiratory tract infections, typical bronchiolitis (with crepitations but no wheeze), or RSV-induced wheezy bronchitis. It has been suggested that RSV-associated wheezy bronchitis, but not typical bronchiolitis, might be associated with subsequent atopy and asthma (8, 9). On the other hand, infections by other viruses, particularly rhinoviruses, are followed by recurrent wheeze (10), suggesting that the host response, rather than the type of virus, predicts later outcome.

Asthma phenotype was poorly defined in both studies. There is much evidence suggesting that there are several phenotypes of early childhood wheezing disease (11, 12). Using the old umbrella term "asthma," which ignores this heterogeneity, may be misleading, particularly when investigating pathways and causation. In the Denmark study, asthma was defined as a discharge diagnosis of asthma at any time, or a parent report of "asthma ever in life" collected at the age of 3 to 5 years. Another recently published report by Thomsen and colleagues, which analyzed causality by applying a different statistical approach (time to event analysis) to the same twin dataset, reveals that a large proportion of these "asthma" hospitalizations occurred in 1-year olds and that the increased risk of "asthma" disappears by 1 year after RSV infection (13). This suggests that most children with so-called "asthma" might have had recurrent viral wheeze, a largely self-limiting disorder of which RSV lower airway disease may simply be one manifestation. Indeed, the Denmark studies provide further indirect evidence in support of the contention that there are several phenotypes of early childhood wheezing disease. Cohort studies assessing asthma outcomes at different ages have in fact suggested that the association between RSV infections and later wheeze wanes with time, and that there is no association with atopy (14, 15).

To summarize, we think these new data are more in favor of the hypothesis that the association between RSV and asthma is due to shared predisposition rather than to a causal effect of RSV. However, clearer phenotype definitions both of early childhood viral disease ("bronchiolitis") and of subsequent recurrent wheezing disorders ("asthma") are absolute requisites for pro-

gress in discovering the pathways leading to childhood asthma. Without specifying what disorders we are dealing with, this type of epidemiological or genetic research will remain inconclusive.

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