Published in final edited form as:

Future Virol. 2015 July ; 10(7): 883–897. doi:10.2217/fvl.15.55.

Genes associated with RSV lower respiratory tract infection and asthma: the application of genetic epidemiological methods to understand causality

Emma K Larkin^{1,*} and Tina V Hartert¹

¹Department of Medicine, Division of Allergy, Pulmonary & Critical Care Medicine, Vanderbilt University Medical Center, Nashville, TN 37232, USA

Abstract

Infants with respiratory syncytial virus (RSV) lower respiratory tract infections (LRIs) are at increased risk for childhood asthma. The objectives of this article are to review the genes associated with both RSV LRI and asthma, review analytic approaches to assessing shared genetic risk and propose a future perspective on how these approaches can help us to understand the role of infant RSV infection as both an important risk factor for asthma and marker of shared genetic etiology between the two conditions. The review of shared genes and thus pathways associated with severity of response to RSV infection and asthma risk can help us to understand mechanisms of disease and ultimately propose new and novel targets for primary prevention of both diseases.

Keywords

asthma; bronchiolitis; genome-wide association study; human genetics; infant; lower respiratory tract infection; respiratory syncytial virus; review; wheezing

More than half of infants experience respiratory syncytial virus (RSV) infection during their first winter virus season; roughly two-thirds of these infections are upper respiratory tract infections (URIs) and the remaining a third are lower respiratory tract infections (LRIs). RSV is the most common cause of infant hospitalization, and one of the most common causes of infant mortality worldwide [1–10]. RSV is responsible not just for infant morbidity, but infants with RSV bronchiolitis or LRI requiring hospitalization have a very high likelihood of developing childhood asthma [11–18]. Between 16 and 48% of infants hospitalized for RSV bronchiolitis/LRIs are later diagnosed with childhood asthma, with estimates varying by length of follow-up [12–14,18–20]. There is now convincing evidence from a number of cohorts that RSV LRI and other viral wheezing illnesses, regardless of hospitalization status, are significant, independent risk factors for later wheezing, at least

* Author for correspondence: Tel.: +1 615 936 2723; emma.larkin@vanderbilt.edu.

For reprint orders, please contact: reprints@futuremedicine.com

Financial & competing interests disclosure

This study was supported by National Institute of Allergy and Infectious Diseases grant U19-AI-095277 and NIH K24 AI-77930. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

within the first decade of life [12,13,21–28]. While the association between viral LRI during infancy and asthma is well established, it remains to be determined whether severe respiratory infection causes asthma and/or serves as a marker for those infants genetically predisposed to develop asthma. We believe that infant RSV LRI is both a marker of those genetically predisposed to develop childhood asthma, representing their first wheezing illness, and an active participant in the causal pathway between host genetics and asthma. This article first reviews the studies that have been conducted on both RSV and asthma, and identifies those that are shared by both diseases, focusing on the concept of shared genetic risk and how genetic epidemiologic methods can be used to identify and quantify genetic underpinnings of both diseases.

Asthma is a common, chronic and complex disease with genetic and environmental components. Asthma is a public health problem for all countries regardless of level of development [4–6,8,29]. Asthma has a strong genetic component with heritability estimates ranging from 25 to 95% (most estimates in the 60–70% range) [30–37]. Many environmental factors have also been shown to be associated with asthma including: primary/secondary tobacco smoke, farm animals, household pets, antibiotic/acetaminophen use, breastfeeding, caesarean delivery, nutrition, air pollution, allergens and viral infections [38].

The wide range of heritability estimates and lengthy list of environmental exposures reflect the fact that ‘asthma’ is not a homogenous disease and there are various asthma phenotypes, which include atopic, intrinsic, extrinsic, adult-onset, early onset and others. Because the attributable risk of childhood asthma in infants who have had an LRI event during RSV season is estimated at 47–49%, the primary phenotype we are considering is childhood asthma, following RSV exposure in infancy. Prior to age 6, allergic sensitization precedes and is a risk factor for asthma. There are also intermediate wheezing illness phenotypes, such as transient early wheezing, late onset wheezing and persistent or recurrent wheezing that can be considered as preasthma phenotypes and incorporated into a generalized phenotype called childhood wheezing illnesses [39]. Wheezing illnesses is an unstandardized term as research cohorts have defined early preschool wheezing outcomes based on alternative frequencies of occurrence and cohort-specific ages. Throughout childhood, symptoms of asthma may remit or worsen [40]. Although this review focuses on RSV during the first year of life, we recognize the human rhinovirus (HRV) is also an important source of viral exacerbations during early childhood, as well.

Multiple lines of evidence support a direct causal role of RSV infection on asthma and wheezing illnesses, [41] independent of host genetics. Ecological evidence demonstrates increasing rates of both diseases within the same population [25]. Moreover, multiple association studies have not only consistently demonstrated a strong association between infant bronchiolitis severity and asthma or wheezing illness risk but also a large effect size and a dose-dependent relationship [18,27,42–44]. Open-label studies of RSV immunoprophylaxis have demonstrated that those who received preventive therapy have decreased rates of early childhood wheezing and asthma [45–47]. Lastly, a recent randomized clinical trial of RSV immunoprophylaxis demonstrated that prevention of RSV infection decreased 1-year wheezing outcomes in late preterm infants [48]. The

randomization process likely ensured balanced genetic risk to both RSV LRI and wheezing in treatment and control groups suggesting a direct causal role of infant RSV infection in wheezing illnesses. Follow-up will require future research to extend results to childhood asthma. RSV is known to cause lung injury, chronically diminishing lung function throughout childhood and causing persistent airway hyperreactivity [49–51]. Additionally, there are significant parallels between the host response to RSV that are similar to the immunologic profile and histologic changes seen in asthma. Among infants with severe RSV infection, the proinflammatory cytokine TNF- α was elevated compared with non-ill infants [52]. TNF- α may be important in long-term inflammation and lung remodeling as found in neonatal mouse models of RSV [53]. Other inflammatory cytokines including sICAM1 and IL-10 measured in infant nasal wash and serum IL-6 correlate with the severity of illness [54]. After infant RSV LRI, cysteinyl leukotrienes remain elevated in nasal lavage fluid at time of infection and 1 month later, a very important mediator of wheezing and asthma [55]. In an animal model, another paramyxovirus infection, Sendai virus, results in acute and chronic epithelial and airway reactivity changes that are nearly identical to human asthma [56].

Not all infants who are exposed to RSV develop LRI and not all infants with RSV LRI develop childhood asthma, suggesting that additional factors, such as host genetic background, are important in disease pathophysiology. Several studies help explain how RSV, as an environmental insult operating under a specific genetic background, may cause asthma or early childhood wheezing illnesses. A compelling study shows that repeated RSV infection in mice previously tolerized to ovalbumin through breast milk induced a Th2-like inflammation environment in the lung and influenced regulatory T cells to obtain a Th2 effector phenotype that has a decreased ability to suppress a response to unrelated antigens [57]. This supports a previous study in which Sendai viral (also a paramyxovirus) infection increased Th2 polarization, suggesting that the host response can be ‘sensitized’ toward allergic responses against other allergens [58]. Here, gain-of-function variants in IL-4 and IL-13, and many other allergenic genes, would enhance the odds of developing severe RSV disease and asthma in infected children. Consistent with this finding, more severe RSV disease leads to more severe chronic lung disease [12,13,22,26,59], and individuals with loss-of-function polymorphisms in antioxidant genes [60] or exaggerated inflammatory responses [25,61–64] would be at increased risk of RSV-induced chronic lung disease. RSV F protein is a strong agonist of the pattern recognition receptor: TLR4. TLR4 regulation in the respiratory epithelium mediates inception of asthma caused by house dust mites, a frequent exacerbator of childhood allergic asthma [65].

One challenge in identifying the genetic variants responsible for childhood asthma is that specific genetic risks may only be important in combination with specific environmental exposures [66] such as viral infection, which may act during crucial developmental time points in infancy. Infants under 6 months of age are at increased risk of LRI, compared with infants over the age of 6 months. The risk of LRI due to RSV exposure is highest between ages 2 and 4 months, possibly due to waning maternal antibodies, and not fully developed lungs and immune system [67,68].

First, we review evidence from both single nucleotide polymorphisms (SNPs) analyses, and available genome-wide studies for both RSV and asthma, and demonstrate shared risk genes for both conditions (Figure 1). Second, we show how whole genome approaches (e.g., heritability and pathway analyses) that aggregate the effects of genetic variants or SNPs across the entire genome can answer questions about causality. Lastly, we identify the gaps in our knowledge and approaches to addressing critical remaining questions.

Status of current genetic knowledge on shared genes for RSV & asthma

There are genes that have been identified to be associated with both severity of RSV infection and asthma. Below we summarize the state of the field in asthma and early-life RSV infection while emphasizing the genes that overlap in both disease processes. This is not a meta-analysis or systematic review of genes for either disease, which can be found elsewhere [38,69–73]. Here, we emphasize how genes for each disease overlap. Genes selected for asthma are based on prior reviews of candidate genes for asthma [38,73,74] plus a download of any asthma phenotype from NHGRI catalog using an expansive definition of asthma to capture as many genetic variants as possible that overlap with RSV LRI. A comprehensive review of the genetics of asthma can be found in [73]. Candidate genes for RSV LRI were selected for inclusion based on prior reviews [69,71,72,75] plus any new research published since then, based on PubMed searches containing terms ‘RSV genetics human SNP,’ along with related citations. By design, we do not exclude any null studies to identify all potential overlap and emphasize the point that genetic associations may not be observed in the absence of exposure to RSV.

The genetic basis of asthma has a lengthy history with many heritability factors identified prior to genome-wide association studies (GWAS), including family aggregation and heritability studies, linkage analyses and candidate gene studies that have demonstrated significant replicated associations [73]. More recently, many of these large-scale GWAS have been performed on the spectrum of asthma phenotypes in diverse populations [76–104], identifying genetic associations with asthma. While both candidate gene and GWAS have been successful in identifying and replicating associations, very few have been proven to be causal, reflecting a polygenic inheritance pattern and environmental components. GWAS offer an unbiased approach to gene discovery compared with candidate gene approaches, but the two approaches differ in how they decide upon meaningful associations. For example, the two approaches rely on different p-value thresholds that reflect variable effect sizes in different populations, and they also often use different phenotypic definitions of asthma. In Figure 2, we present genetic variants associated with asthma, incorporating evidence from both candidate gene and GWAS, recognizing that not all of the genetic risk variants have the same evidence for association. The blue region of the Venn diagram shows that GWAS have identified over 80 significant genes associated with asthma or as part of a significant interaction with another factor related to asthma at $p < 10^{-5}$. In addition, over 80 SNPs have been identified through replicated candidate gene studies – but very few of genes overlap between the two, likely due to different standards of significance.

Compared with asthma, there are very few genetic studies of severity or susceptibility to RSV infection and none have been performed unbiasedly through GWAS, despite the

availability of affordable dense genotyping arrays. The candidate gene studies performed are hypothesis driven and chosen based on meaningful biologic pathways. This approach has the advantage of reducing the number of multiple comparisons, thus the threshold of significance is substantially higher (or more liberal) than what is utilized in GWAS. The most comprehensive study of RSV LRI genetics was conducted by Janssen *et al.* using 384 polymorphisms from 220 genes selected from biologically meaningful pathways: Airway mucosal response, innate immunity, adaptive immunity, chemotaxis and known allergic asthma genes [105]. Their main finding was that genes from innate immune pathway are important in RSV infection severity [105].

Because RSV infection is associated with asthma inception, and as asthmatics have increased susceptibility to certain viral and bacterial pathogens, it makes sense that genes may associate with both diseases. The genes associated with asthma and RSV, and those that are shared by both diseases are listed in Figure 2 as a Venn diagram [59,69,105–123]. The genes in the overlapping purple circle in Figure 2 are those genes with at least one significant association ($p < 0.05$) that are associated with both RSV and asthma or wheezing illnesses, where the evidence of an association with asthma is strongly supported.

Some research has already been performed to answer important questions about which children develop wheezing illnesses after LRI. One study demonstrated significant associations with wheezing in the 15 months after RSV LRI (*IL19*, *IL20*, *MUC5AC*, *TNFRSF1B*, *C3*, *CTLA4*, *CXCL9*, *IL4R* and *IL7*) [124], of which *C3*, *CTLA4* and *IL4R* overlap with variants identified with asthma [125–129]. They also identified a functional SNP in *IL13* associated with 6-year wheezing outcomes ($n = 72$) after RSV LRI [130], which has replicated inconsistently in studies of asthma [125]. If RSV LRI is on the causal pathway to asthma, the association between an RSV infection and asthma may not be replicated, depending on the presence and timing of an RSV environmental exposure. In the blue region, we show asthma genes that have either show no association with RSV LRI severity or have not been studied in RSV. We also indicate in the pink region the genes which are associated with RSV infection with an unconfirmed association with asthma.

The lack of any GWAS for RSV infection severity means that many SNPs, studied extensively in asthma have not been examined in RSV exposure studies. Because GWASs provide widespread coverage of the genome for asthma, many of the SNPs identified for RSV infection severity may have some association with childhood asthma, but are too weak to reach statistical significance (as listed in the pink region of Figure 2). Published catalogs [123] or published online supplements are limited to study specific statistical thresholds; thus, it is impossible to distinguish SNPs with no known association with asthma, compared with SNPs with weak associations with asthma. SNPs of weak effect sizes are believed to constitute the missing heritability of a trait, that is, responsible for the additional phenotypic variance that is unexplained by multiple comparisons corrected significant SNP hits [131]. Of course, phenotype misclassification, gene–gene interactions, gene–viral interactions, exposure to other asthma risk factors and an early-life RSV environmental insult can also explain the missing heritability.

If RSV exposure is on the causal pathway to asthma, the association between RSV severity and asthma may not be replicated, especially if there is a critical window during which infant RSV infection is associated with greatest risk of asthma. The challenge is that some children who go on to develop asthma may never have been exposed to RSV infection in early infancy and children with RSV infection may have a mild or severe response likely based in part on genetic factors. Determining early-life exposure to RSV infection requires detailed viral surveillance in the first year of life. Thus, achieving adequate sample sizes of infants with well-characterized infant RSV exposure will require multicohort collaborations.

Review of statistical approaches that could be used to test the relationship between RSV LRI & asthma from GWAS

One of the most time- and cost-efficient ways to proceed with dissecting the relationship between RSV LRI and future childhood asthma is through genome-wide genotyping with array products that can allow over 4.3 million SNPs or much more, if imputation procedures for missing SNPs are included. While not as in-depth as whole sequencing or exome sequencing, standard GWAS arrays can provide invaluable information that is unbiased with respect to candidate gene studies. Moreover, emerging statistical techniques make it possible to study the relationship between asthma and RSV infection severity in independent populations, through genetic correlation studies, which also require that each disease has a heritable component. Previously, the broad sense heritability estimate for a severe RSV phenotype: hospitalization for acute RSV infection, which includes dominant and additive genetic components, was between 16 and 20% based on administrative data on twins [132], under the reasonable assumption that if one twin is exposed to RSV, the other twin is exposed as well. For the heritability estimate to be unbiased, it is further assumed that hospitalization decisions for each twin are determined separately, which is unlikely the case. While hospitalization for RSV infection captures a severe phenotype of RSV infection, additional relevant phenotypes for study may include LRI severity as measured by an ordinal severity scale [133] or a dichotomous LRI present or absent regardless of whether treated in a hospital, emergency room or outpatient visit.

The biggest factor in estimating heritability and genetic correlations between RSV infection severity and asthma is the appropriate selection of a control population. For example, the ideal control population is one with confirmation of RSV exposure, but without severe illness. Risk of RSV infection is time dependent and because of the seasonality of RSV circulation, not all infants are exposed to RSV during the first months of life when primary infection is most likely to result in severe disease. This can decrease the observed genetic relationship based on shared genetics of RSV LRI and asthma because of exposure dependency. Tests for antibodies to RSV infection require serological testing for RSV, ideally, after RSV has ceased circulating.

Heritability of RSV infection severity

Over the past 5 years, statistical methods have been developed which make it possible to estimate the heritability of RSV LRI as a binary trait, using generalized linear-mixed modeling (GLMM) based on genome-wide data, which have been implemented in the

program Genetic Complex Trait Analysis (GCTA) [134]. This can be done using Falconer's threshold liability model for a disease [135], which assumes that the underlying scale of disease liability follows standard normal distribution and that a binary trait can be linearly transformed to the unobserved continuous scale [136] to estimate the proportion of the variance (heritability of the liability). The first step in this analysis is to estimate the genetic relatedness matrix (GRM) of all pairs of infants in a case and control population to determine their similarity across the entire genome based on the products of the indicator variables for the genotypes divided by the heterozygosity of each SNP and averaged across all SNPs in the genome [134,136]. The next step is to estimate the variance explained by all autosomal SNPs by restricted maximum likelihood analysis of a mixed linear effects model. The proportion of the variance and its standard error explained by all autosomal SNPs can be estimated as the heritability through the equation: $h^2_G = \sigma_G^2 / \sigma_P^2$, where σ_P^2 is the total phenotypic variance and σ_G^2 is the additive genetic variance. This heritability estimate represents the lower bound of true heritability in the narrow sense due to the fact that the genotyped markers may only be in linkage disequilibrium with causal variants [137]. Currently, GCTA only considers the additive genetic variance and not variation due to dominance, gene–gene interactions and a common environment (i.e., nonadditive genetic variance), which are usually included in twin studies of heritability. The GCTA approach has been successfully used in analyses of heritability of height, body mass index, diabetes, schizophrenia and prolonged QT interval [137–139].

Genetic correlation

The degree of genetic correlation between severe RSV infection and childhood asthma can be measured (Figure 1: group C representing the overlap of groups A and B). Genetic correlation measures how much of the genetic influence of two traits is overlapping or shared. Therefore, it is possible to quantify the extent to which infant RSV infection serves as a surrogate of shared genes associated with both severe RSV infection and asthma, and/or the extent to which RSV infection plays a causal role in asthma development. This approach uses a bivariate linear-mixed model as implemented in GCTA to estimate the genetic correlation between asthma and the presence of RSV LRI versus URI, restricted to individuals who are RSV positive. The heritability model described in the last paragraph can be extended to allow for a variance covariance matrix of two traits, then can be written as a function of the GRM, the additive genetic variance (σ_g^2), residual variance (σ_e^2) and covariance between the genetic variances of the two traits (σ_{g1g2}^2). This equation is spelled out in full elsewhere [139,140]. The genetic correlation is calculated as $\sigma_{g1g2}^2 / (\sigma_{g1}^2 \sigma_{g2}^2)$, with numbers indexing each trait. It has been shown that the correlation on the scale of liability is similar to the observed discrete scale [140]. The elegance of genetic correlations is that the genetic variances for RSV LRI can be measured in one population with RSV positive (ideally) URI as controls; while asthma cases and controls can be measured in another population. Theoretically, this can be done in large DNA repositories linked to electronic medical records, if large enough sample sizes are available. It would be statistically more powerful to estimate genetic correlations with two phenotypes (infant RSV infection severity and asthma) in the same population, although this would require either extensive surveillance with RSV testing of nasal-pharyngeal samples during RSV season or serologic testing for RSV antibodies with long-term follow-up.

Assessment of genetic correlations directly addresses the extent to which severe RSV infection causes childhood asthma and/or serves as surrogate of shared genetic background between the two traits. This knowledge could be important in designing primary prevention strategies for both RSV and asthma. However, GLMMs can also be used to partition the shared genetic risk between two traits in a pathway-dependent manner. For example, it would be possible to determine to what extent genes in an innate immunity pathway are shared or any other functional grouping. Tests of SNP groups or genes within pathways can become increasingly small in testing, down to a single SNP and mimic a GWAS; however, computational speed can limit the use of GLMM for a GWAS. Such pathway approaches consider the additive role of SNPs of weak effect sizes across biologically known pathways.

HRV wheezing during early childhood is strongly associated with asthma development [24,141]. HRV is also the most common virus associated with disease exacerbations among children with prevalent disease [142]. The GCTA framework makes it possible to compare the relative magnitude of the shared genetic basis of RSV LRI and childhood asthma with that of HRV LRI. It has been shown that the increased risk associated with HRV LRI and childhood asthma is greater than the risk of RSV LRI in children up to 2 years of age. This stronger association between HRV and childhood asthma may be explained by stronger shared genetic underpinnings compared with RSV LRI's shared genetic risk with childhood asthma. A formal statistical test comparing HRV's versus RSV's genetic correlation is testable in the GLMM framework.

Mediation approach & predictive modeling for identification of the shared genes between RSV severity & childhood asthma

It is possible to determine whether the relationship between each SNP and asthma outcomes is mediated by the severity of RSV infection using a GWAS with logistic regression modeling, adjusting for covariates such as gestational age, exposure to secondhand smoke, viral strain or others. This can be done by examining the comparative change in the magnitude of the beta coefficient for each SNP coded additively, and asthma, after adding a binary RSV severity measure (RSV LRI/RSV URI) to the model. This mediation approach, shown in Figure 3, tests a model where allelic variations of genes involved in response to viral infection might confer a predisposition or resistance to both infant LRI and childhood asthma. A multistep mediation analysis includes: regression analysis of asthma on each SNP adjusting for other known risk factors (no RSV LRI vs RSV URI mediator), regression analysis of asthma on RSV LRI versus RSV URI (the mediator) adjusting for other known risk factors and regression analysis of the SNP on asthma adjusting for RSV LRI/RSV URI – the proposed mediator – and other known risk factors. If all three models show significance, then the size of indirect effect (with confidence intervals and significance testing) of RSV LRI as a mediator can be performed by bootstrapping techniques and joint tests of significance. Similarly, the RSV LRI/RSV URI mediator can be considered as an ordinal scale. This approach allows us to estimate direct and indirect effects of each SNP on the causal path to asthma and to identify which SNPs are in the shared pathway of RSV infection and asthma (Figure 1: group C).

An alternative approach is to use a polychotomous logistic model that considers: RSV LRI/asthma, RSV LRI/no asthma, RSV URI/asthma and RSV URI/no asthma as multiple outcomes, adjusting for covariates. Buried within this statistical approach is the concept of prediction modeling, the goal of which is to determine which infants will develop severe infant RSV infection, or childhood asthma following infant RSV infection. The concept of disease prediction underlies personalized medicine, where we can eventually use the genome to identify high-risk groups for disease prevention or tailor therapies for optimal therapeutic effect.

Gene by environment interactions in childhood asthma inception

Mediation models, tested by regression modeling, address the questions about whether RSV LRI affects risk of asthma and whether the same genetic risk factors underlie both conditions [70,143–145]. An alternative modeling framework is to consider effect modification by RSV exposure on the genetic relationship between a SNP and childhood asthma, also known as gene by environment (GxE) interactions. What distinguishes this type of analytic approach is that it can incorporate no exposure to RSV during infancy into the model, recognizing that exclusion of RSV exposure requires accurate confirmation of no RSV infection at the end RSV season. It is also possible to include the presence and absence of RSV exposure in a GxE interaction model, where RSV exposure is an effect modifier. This can be done through logistic regression modeling, where the outcome is childhood asthma. This modeling includes: a SNP as a main effect, RSV infection severity (RSV LRI, RSV URI and no RSV exposure), interaction terms between SNPs and RSV infection severity, and relevant covariates.

Examples of environmental factors that modify the expression of genes for asthma (GxE interaction) abound in the literature such as, second-hand smoke, ambient air pollutants, aeroallergen and endotoxin exposure [146–152]. Most of these interactions have been identified in the context of candidate gene studies [152]. In the first genome-wide interaction study of asthma with farm exposure in Western Europe as an effect modifier, the top gene identified was GRM1 ($p = 0.0003$), which did not meet genome-wide significance [153]. A more recent genome-wide interaction study of childhood asthma with discovery and replication stages, identified a genetic interaction with *in utero* second-hand smoke exposure, although the discovery sample did not meet the genome-wide significance threshold (EPB41L3; $p = 4.29 \times 10^{-5}$) [154]. Not every interaction has replicated, for many reasons, including small sample sizes, variable linkage disequilibrium of the tested polymorphisms with causal variants or varying definitions of the environmental exposure. Daycare exposure, a possible surrogate measure of exposure to viruses, has been studied as an effect modifier on the relationship between genetic polymorphisms and cytokine profiles and/or atopic outcomes, noting that neither factor was significantly associated with atopic phenotypes alone [155]. The genetic variant and daycare attendance exposures were both required for these associations to be observed [155], supporting the idea that genetic risk variants and environmental exposures, such as viruses, may be required to affect risk of common diseases such as asthma [156].

Clinical implications of studies of shared genetic risk

Knowing the extent to which RSV infection severity is causal or a marker of shared genetic risk with wheezing and asthma outcomes means we can potentially optimize ways to prevent asthma or reduce its morbidity [41]. For example, we could potentially identify infants who would benefit from prevention of RSV LRI by immunoprophylaxis with monoclonal antibodies based on a genetic risk score. Similarly, in a different risk profile group, we may be able to lessen the burden of wheezing illnesses in an infant who has already had an RSV LRI, through aggressive follow-up and treatment. While this type of personalized medicine continues to develop and advance, research into new therapies can expand with knowledge about important pathways derived from genetic studies.

Conclusion

This review article summarizes what has been done to understand the genetics of asthma and severity of infant RSV infection as individual diseases. We discuss how little the genetic findings overlap, due primarily to the lack of unbiased genotyping in RSV infection or different statistical thresholds used in different study designs. We propose the application of genetic epidemiologic methods built on genome-wide genotyping that can be used to address the different relationships between severe RSV infection and asthma risk, including common genetic factors, effect modification and mediation. Achieving adequate sample sizes for these models will require collaborations between cohorts with detailed viral surveillance in the first year of life. While infant RSV infection may act as a causal agent in asthma development, it is also likely that there is a shared genetic predisposition common to both diseases. This knowledge, while helping us to prevent disease, is likely to additionally be helpful in understanding the mechanisms through which viruses exacerbate prevalent asthma. New statistical methods make it possible to draw inferences and make estimates about the shared genetic liability for asthma and LRI in different populations that have genome-wide genotyping. However, individual genetic risk prediction requires detailed information from infant populations with known RSV exposure during infancy who are followed to childhood asthma.

Future perspective

Identification of shared genetic risk factors for two correlated traits requires the availability of unselected genotyping data, which exist in abundance for asthma outcomes, but not for RSV LRI. The new genetic epidemiologic approaches that rely on GWAS data make it possible to estimate the shared genetic risk between RSV infection and wheezing asthma, based on independent populations. This means that large-scale genotyping efforts in large DNA repositories with corresponding electronic medical records may be able to effectively utilize existing data with large samples sizes to make inferences about important causal pathways for each disease separately or jointly. Until genomic prediction methodologies are fully developed, data from individuals followed longitudinally for many years will be needed to determine individual genetic risk for childhood asthma following infant RSV LRI. This will require taking advantage of existing research cohorts or DNA repositories

linked to phenotype data from stable populations with lengthy follow-up of the same individuals.

References

Papers of special note have been highlighted as:

- of interest;
- of considerable interest

1. Glezen WP, Taber LH, Frank AL, et al. Risk of primary infection and reinfection with respiratory syncytial virus. *Am J Dis Child*. 1986; 140:543–546. [PubMed: 3706232]
2. Shay DK, Holman RC, Newman RD, et al. Bronchiolitis-associated hospitalizations among US children, 1980–1996. *JAMA*. 1999; 282:1440–1446. [PubMed: 10535434]
3. Shay DK, Holman RC, Roosevelt GE, et al. Bronchiolitis-associated mortality and estimates of respiratory syncytial virus-associated deaths among US children, 1979–1997. *J Infect Dis*. 2001; 183:16–22. [PubMed: 11076709]
4. WHO. Prevention of Allergy and Allergic Asthma. WHO; Geneva, Switzerland: 2003.
5. Asher MI, Montefort S, Bjorksten B, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC phases one and three repeat multicountry cross-sectional surveys. *Lancet*. 2006; 368:733–743. [PubMed: 16935684]
6. Eder W, Ege MJ, von ME. The asthma epidemic. *N Engl J Med*. 2006; 355:2226–2235. [PubMed: 17124020]
7. Leader S, Kohlhasse K. Respiratory syncytial virus-coded pediatric hospitalizations, 1997 to 1999. *Pediatr Infect Dis J*. 2002; 21:629–632. [PubMed: 12237593]
8. Maziak W, Behrens T, Brasky TM, et al. Are asthma and allergies in children and adolescents increasing? Results from ISAAC phase I and phase III surveys in Munster, Germany. *Allergy*. 2003; 58:572–579. [PubMed: 12823113]
9. Carroll KN, Gebretsadik T, Griffin MR, et al. Increasing burden and risk factors for bronchiolitis-related medical visits in infants enrolled in a state health care insurance plan. *Pediatrics*. 2008; 122:58–64. [PubMed: 18595987]
10. Hall CB, Weinberg GA, Iwane MK, et al. The burden of respiratory syncytial virus infection in young children. *N Engl J Med*. 2009; 360:588–598. [PubMed: 19196675]
11. Wittig HJ, Glaser J. The relationship between bronchiolitis and childhood asthma: a follow-up study of 100 cases of bronchiolitis. *J Allergy*. 1959; 30:19–23. [PubMed: 13620441]
12. Henderson J, Hilliard TN, Sherriff A, et al. Hospitalization for RSV bronchiolitis before 12 months of age and subsequent asthma, atopy and wheeze: a longitudinal birth cohort study. *Pediatr Allergy Immunol*. 2005; 16:386–392. [PubMed: 16101930]
13. Sigurs N, Bjarnason R, Sigurbergsson F, et al. Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. *Am J Respir Crit Care Med*. 2000; 161:1501–1507. [PubMed: 10806145]
14. Sigurs N, Gustafsson PM, Bjarnason R, et al. Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. *Am J Respir Crit Care Med*. 2005; 171:137–141. [PubMed: 15516534]
15. Kotaniemi-Syrjanen A, Vainionpaa R, Reijonen TM, et al. Rhinovirus-induced wheezing in infancy – the first sign of childhood asthma? *J Allergy Clin Immunol*. 2003; 111:66–71. [PubMed: 12532098]
16. Noble V, Murray M, Webb MS, et al. Respiratory status and allergy nine to 10 years after acute bronchiolitis. *Arch Dis Child*. 1997; 76:315–319. [PubMed: 9166022]
17. Murray M, Webb MS, O’callaghan C, et al. Respiratory status and allergy after bronchiolitis. *Arch Dis Child*. 1992; 67:482–487. [PubMed: 1580676]
18. Bacharier LB, Cohen R, Schweiger T, et al. Determinants of asthma after severe respiratory syncytial virus bronchiolitis. *J Allergy Clin Immunol*. 2012; 130:91–100. [PubMed: 22444510]

19. Pullan CR, Hey EN. Wheezing, asthma, and pulmonary dysfunction 10 years after infection with respiratory syncytial virus in infancy. *Br Med J (Clin Res Ed)*. 1982; 284:1665–1669.
20. James KM, Gebretsadik T, Escobar GJ, et al. Risk of childhood asthma following infant bronchiolitis during the respiratory syncytial virus season. *J Allergy Clin Immunol*. 2013; 132(1): 227–229. [PubMed: 23419541]
21. Martinez FD, Wright AL, Taussig LM, et al. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. *N Engl J Med*. 1995; 332:133–138. [PubMed: 7800004]
22. Weber MW, Milligan P, Giadom B, et al. Respiratory illness after severe respiratory syncytial virus disease in infancy in the Gambia. *J Pediatr*. 1999; 135:683–688. [PubMed: 10586169]
23. Kusel MM, de Klerk NH, Keadze T, et al. Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. *J Allergy Clin Immunol*. 2007; 119(5):1105–1110. [PubMed: 17353039]
24. Jackson DJ, Gangnon RE, Evans MD, et al. Wheezing rhinovirus illnesses in early life predict asthma development in high risk children. *Am J Respir Crit Care Med*. 2008; 178(7):667–672. [PubMed: 18565953]
25. Wu P, Dupont WD, Griffin MR, et al. Evidence of a causal role of winter virus infection during infancy on early childhood asthma. *Am J Respir Crit Care Med*. 2008; 178:1123–1129. [PubMed: 18776151]
26. Stein RT, Sherrill D, Morgan WJ, et al. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. *Lancet*. 1999; 354:541–545. [PubMed: 10470697]
27. Lemanske RF Jr, Jackson DJ, Gangnon RE, et al. Rhinovirus illnesses during infancy predict subsequent childhood wheezing. *J Allergy Clin Immunol*. 2005; 116:571–577. [PubMed: 16159626]
28. Stensballe LG, Kristensen K, Simoes EA, et al. Atopic disposition, wheezing, and subsequent respiratory syncytial virus hospitalization in Danish children younger than 18 months: a nested case–control study. *Pediatrics*. 2006; 118:e1360–e1368. [PubMed: 17079537]
29. Worldwide variations in the prevalence of asthma symptoms: the International Study of Asthma and Allergies in Childhood (ISAAC). *Eur Respir J*. 1998; 12:315–335. [PubMed: 9727780]
30. van Beijsterveldt CE, Boomsma DI. Genetics of parentally reported asthma, eczema and rhinitis in 5-yr-old twins. *Eur Respir J*. 2007; 29:516–521. [PubMed: 17215318]
31. Duffy DL, Martin NG, Battistutta D, et al. Genetics of asthma and hay fever in Australian twins. *Am Rev Respir Dis*. 1990; 142:1351–1358. [PubMed: 2252253]
32. Koeppen-Schomerus G, Stevenson J, Plomin R. Genes and environment in asthma: a study of 4 year old twins. *Arch Dis Child*. 2001; 85:398–400. [PubMed: 11668102]
33. Tan H, Walker M, Gagnon F, et al. The estimation of heritability for twin data based on concordances of sex and disease. *Chronic Dis Can*. 2005; 26:9–12. [PubMed: 16117840]
34. Fagnani C, Annesi-Maesano I, Brescianini S, et al. Heritability and shared genetic effects of asthma and hay fever: an Italian study of young twins. *Twin Res Hum Genet*. 2008; 11:121–131. [PubMed: 18361712]
35. Willemsen G, van Beijsterveldt TC, van Baal CG, et al. Heritability of self-reported asthma and allergy: a study in adult Dutch twins, siblings and parents. *Twin Res Hum Genet*. 2008; 11:132–142. [PubMed: 18361713]
36. Thomsen SF, van der Sluis S, Kyvik KO, et al. Estimates of asthma heritability in a large twin sample. *Clin Exp Allergy*. 2010; 40:1054–1061. [PubMed: 20528882]
37. Thomsen SF, van der Sluis S, Kyvik KO, et al. Increase in the heritability of asthma from 1994 to 2003 among adolescent twins. *Respir Med*. 2011; 105:1147–1152. [PubMed: 21450446]
38. Ober C, Yao TC. The genetics of asthma and allergic disease: a 21st century perspective. *Immunol Rev*. 2011; 242:10–30. Excellent review of the genetics of asthma, although Mathias [73] is more recent. [PubMed: 21682736]
39. Stein RT, Holberg CJ, Morgan WJ, et al. Peak flow variability, methacholine responsiveness and atopy as markers for detecting different wheezing phenotypes in childhood. *Thorax*. 1997; 52:946–952. [PubMed: 9487341]
40. Wu P, Carroll KN, Gebretsadik T, et al. The developmental trajectory of pediatric asthma in 3- to 10-year-olds. *J Allergy Clin Immunol*. 2012; 129:1397–1398. [PubMed: 22322007]

41. Feldman AS, He Y, Moore ML, et al. Toward primary prevention of asthma. Reviewing the evidence for early-life respiratory viral infections as modifiable risk factors to prevent childhood asthma. *Am J Respir Crit Care Med*. 2015; 191:34–44. [PubMed: 25369458]
42. Escobar GJ, Ragins A, Li SX, et al. Recurrent wheezing in the third year of life among children born at 32 weeks' gestation or later: relationship to laboratory-confirmed, medically attended infection with respiratory syncytial virus during the first year of life. *Arch Pediatr Adolesc Med*. 2010; 164:915–922. [PubMed: 20921348]
43. Turner SW, Young S, Landau LI, et al. Reduced lung function both before bronchiolitis and at 11 years. *Arch Dis Child*. 2002; 87:417–420. [PubMed: 12390918]
44. Carroll KN, Wu P, Gebretsadik T, et al. Season of infant bronchiolitis and estimates of subsequent risk and burden of early childhood asthma. *J Allergy Clin Immunol*. 2009; 123:964–966. [PubMed: 19181372]
45. Yoshihara S, Kusuda S, Mochizuki H, et al. Effect of palivizumab prophylaxis on subsequent recurrent wheezing in preterm infants. *Pediatrics*. 2013; 132:811–818. [PubMed: 24127479]
46. Wenzel SE, Gibbs RL, Lehr MV, et al. Respiratory outcomes in high-risk children 7 to 10 years after prophylaxis with respiratory syncytial virus immune globulin. *Am J Med*. 2002; 112:627–633. [PubMed: 12034412]
47. Simoes EA, Groothuis JR, Carbonell-Estrany X, et al. Palivizumab prophylaxis, respiratory syncytial virus, and subsequent recurrent wheezing. *J Pediatr*. 2007; 151:34–42. [PubMed: 17586188]
- 48•. Blanken MO, Rovers MM, Molenaar JM, et al. Respiratory syncytial virus and recurrent wheeze in healthy preterm infants. *N Engl J Med*. 2013; 368:1791–1799. This randomized clinical trial for RSV immunoprophylaxis provides compelling evidence that RSV is causal in wheezing illness in the first year of life. [PubMed: 23656644]
49. Hall WJ, Hall CB. Clinical significance of pulmonary function tests. Alterations in pulmonary function following respiratory viral infection. *Chest*. 1979; 76:458–465. [PubMed: 225132]
50. Lukacs NW, Tekkanat KK, Berlin A, et al. Respiratory syncytial virus predisposes mice to augmented allergic airway responses via IL-13-mediated mechanisms. *J Immunol*. 2001; 167:1060–1065. [PubMed: 11441116]
51. Tekkanat KK, Maassab HF, Cho DS, et al. IL-13-induced airway hyperreactivity during respiratory syncytial virus infection is STAT6 dependent. *J Immunol*. 2001; 166:3542–3548. [PubMed: 11207314]
52. Choi J, Callaway Z, Kim HB, et al. The role of TNF-alpha in eosinophilic inflammation associated with RSV bronchiolitis. *Pediatr Allergy Immunol*. 2010; 21:474–479. [PubMed: 20088864]
53. You D, Becnel D, Wang K, et al. Exposure of neonates to respiratory syncytial virus is critical in determining subsequent airway response in adults. *Respir Res*. 2006; 7:107. [PubMed: 16893457]
54. Vieira RA, Diniz EM, Ceccon ME. Correlation between inflammatory mediators in the nasopharyngeal secretion and in the serum of children with lower respiratory tract infection caused by respiratory syncytial virus and disease severity. *J Bras Pneumol*. 2010; 36:59–66. [PubMed: 20209309]
55. Da DL, Callegaro S, Carraro S, et al. Nasal lavage leukotrienes in infants with RSV bronchiolitis. *Pediatr Allergy Immunol*. 2007; 18:100–104. [PubMed: 17338781]
56. Kim EY, Battaile JT, Patel AC, et al. Persistent activation of an innate immune response translates respiratory viral infection into chronic lung disease. *Nat Med*. 2008; 14(6):633–640. [PubMed: 18488036]
- 57•. Krishnamoorthy N, Khare A, Oriss TB, et al. Early infection with respiratory syncytial virus impairs regulatory T cell function and increases susceptibility to allergic asthma. *Nat Med*. 2012; 18(10):1525–1530. Research provides experimental evidence for a causal role of RSV in allergic asthma. [PubMed: 22961107]
58. Stephens R, Randolph DA, Huang G, et al. Antigen-nonspecific recruitment of Th2 cells to the lung as a mechanism for viral infection-induced allergic asthma. *J Immunol*. 2002; 169:5458–5467. [PubMed: 12421921]

59. Stensballe LG, Simonsen JB, Thomsen SF, et al. The causal direction in the association between respiratory syncytial virus hospitalization and asthma. *J Allergy Clin Immunol*. 2009; 123:131–137. [PubMed: 19130934]
60. Cho HY, Imani F, Miller-DeGraff L, et al. Antiviral activity of NRF2 in a murine model of respiratory syncytial virus disease. *Am J Respir Crit Care Med*. 2009; 179:138–150. [PubMed: 18931336]
61. Boyce TG, Mellen BG, Mitchel EF Jr, et al. Rates of hospitalization for respiratory syncytial virus infection among children in medicaid. *J Pediatr*. 2000; 137:865–870. [PubMed: 11113845]
62. Hull J, Thomson A, Kwiatkowski D. Association of respiratory syncytial virus bronchiolitis with the interleukin 8 gene region in UK families. *Thorax*. 2000; 55:1023–1027. [PubMed: 11083887]
63. Hull J, Ackerman H, Isles K, et al. Unusual haplotypic structure of IL8, a susceptibility locus for a common respiratory virus. *Am J Hum Genet*. 2001; 69:413–419. [PubMed: 11431705]
64. Hull J, Rowlands K, Lockhart E, et al. Variants of the chemokine receptor CCR5 are associated with severe bronchiolitis caused by respiratory syncytial virus. *J Infect Dis*. 2003; 188:904–907. [PubMed: 12964123]
65. Hammad H, Chieppa M, Perros F, et al. House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. *Nat Med*. 2009; 15:410–416. [PubMed: 19330007]
66. Ioannidis JP. Non-replication and inconsistency in the genome-wide association setting. *Hum Hered*. 2007; 64:203–213. [PubMed: 17551261]
67. Stensballe LG, Ravn H, Kristensen K, et al. Seasonal variation of maternally derived respiratory syncytial virus antibodies and association with infant hospitalizations for respiratory syncytial virus. *J Pediatr*. 2009; 154:296–298. [PubMed: 19150677]
68. Glezen WP, Paredes A, Allison JE, et al. Risk of respiratory syncytial virus infection for infants from low-income families in relationship to age, sex, ethnic group, and maternal antibody level. *J Pediatr*. 1981; 98:708–715. [PubMed: 7229749]
69. Singh AM, Moore PE, Gern JE, et al. Bronchiolitis to asthma: a review and call for studies of gene-virus interactions in asthma causation. *Am J Respir Crit Care Med*. 2007; 175:108–119. [PubMed: 17053206]
70. Wu P, Dupont WD, Griffin MR, et al. A role for genes and environment in the causal relationship between infant RSV infection and childhood asthma. *Am J Respir Crit Care Med*. 2010; 181:194–195. [PubMed: 20053969]
71. Alvarez AE, Marson FA, Bertuzzo CS, et al. Epidemiological and genetic characteristics associated with the severity of acute viral bronchiolitis by respiratory syncytial virus. *J Pediatr (Rio J)*. 2013; 89:531–543. [PubMed: 24035870]
72. Choi EH, Lee HJ, Chanock SJ. Human genetics and respiratory syncytial virus disease: current findings and future approaches. *Curr Top Microbiol Immunol*. 2013; 372:121–137. [PubMed: 24362687]
- 73••. Mathias RA. Introduction to genetics and genomics in asthma: genetics of asthma. *Adv Exp Med Biol*. 2014; 795:125–155. A recent comprehensive review of genes for asthma. [PubMed: 24162907]
74. Ober C, Hoffjan S. Asthma genetics 2006: the long and winding road to gene discovery. *Genes Immun*. 2006; 7:95–100. [PubMed: 16395390]
75. Wu P, Hartert TV. Evidence for a causal relationship between respiratory syncytial virus infection and asthma. *Expert Rev Anti Infect Ther*. 2011; 9:731–745. [PubMed: 21905783]
76. Anantharaman R, Andiappan AK, Nilkanth PP, et al. Genome-wide association study identifies PERLD1 as asthma candidate gene. *BMC Med Genet*. 2011; 12:170. [PubMed: 22188591]
77. Ferreira MA, McRae AF, Medland SE, et al. Association between ORMDL3, IL1RL1 and a deletion on chromosome 17q21 with asthma risk in Australia. *Eur J Hum Genet*. 2011; 19:458–464. [PubMed: 21150878]
78. Hancock DB, Romieu I, Shi M, et al. Genome-wide association study implicates chromosome 9q21.31 as a susceptibility locus for asthma in mexican children. *PLoS Genet*. 2009; 5:e1000623. [PubMed: 19714205]

79. Himes BE, Hunninghake GM, Baurley JW, et al. Genome-wide association analysis identifies PDE4D as an asthma-susceptibility gene. *Am J Hum Genet.* 2009; 84:581–593. [PubMed: 19426955]
80. Li X, Howard TD, Zheng SL, et al. Genome-wide association study of asthma identifies RAD50-IL13 and HLA-DR/DQ regions. *J Allergy Clin Immunol.* 2010; 125:328–335. [PubMed: 20159242]
81. Mathias RA, Grant AV, Rafaels N, et al. A genome-wide association study on African-ancestry populations for asthma. *J Allergy Clin Immunol.* 2010; 125:336–346. [PubMed: 19910028]
82. Moffatt MF, Kabesch M, Liang L, et al. Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. *Nature.* 2007; 448:470–473. [PubMed: 17611496]
83. Moffatt MF, Gut IG, Demenais F, et al. A large-scale, consortium-based genomewide association study of asthma. *N Engl J Med.* 2010; 363:1211–1221. [PubMed: 20860503]
84. Sleiman PM, Flory J, Imielinski M, et al. Variants of DENND1B associated with asthma in children. *N Engl J Med.* 2010; 362:36–44. [PubMed: 20032318]
85. Torgerson DG, Ampleford EJ, Chiu GY, et al. Meta-analysis of genome-wide association studies of asthma in ethnically diverse North American populations. *Nat Genet.* 2011; 43:887–892. [PubMed: 21804549]
86. Wan YI, Shrine NR, Soler AM, et al. Genome-wide association study to identify genetic determinants of severe asthma. *Thorax.* 2012; 67:762–768. [PubMed: 22561531]
87. Himes BE, Jiang X, Hu R, et al. Genome-wide association analysis in asthma subjects identifies SPATS2L as a novel bronchodilator response gene. *PLoS Genet.* 2012; 8:e1002824. [PubMed: 22792082]
88. Ding L, Abebe T, Beyene J, et al. Rank-based genome-wide analysis reveals the association of ryanodine receptor-2 gene variants with childhood asthma among human populations. *Hum Genomics.* 2013; 7:16. [PubMed: 23829686]
89. Ferreira MA, Matheson MC, Tang CS, et al. Genome-wide association analysis identifies 11 risk variants associated with the asthma with hay fever phenotype. *J Allergy Clin Immunol.* 2014; 133:1564–1571. [PubMed: 24388013]
90. Duan QL, Lasky-Su J, Himes BE, et al. A genome-wide association study of bronchodilator response in asthmatics. *Pharmacogenomics J.* 2014; 14:41–47. [PubMed: 23508266]
91. Du R, Litonjua AA, Tantisira KG, et al. Genome-wide association study reveals class I MHC-restricted T cell-associated molecule gene (CRTAM) variants interact with vitamin D levels to affect asthma exacerbations. *J Allergy Clin Immunol.* 2012; 129:368–373. [PubMed: 22051697]
92. Drake KA, Torgerson DG, Gignoux CR, et al. A genome-wide association study of bronchodilator response in Latinos implicates rare variants. *J Allergy Clin Immunol.* 2014; 133:370–378. [PubMed: 23992748]
93. Ferreira MA, Matheson MC, Duffy DL, et al. Identification of IL6R and chromosome 11q13.5 as risk loci for asthma. *Lancet.* 2011; 378:1006–1014. [PubMed: 21907864]
94. Galanter JM, Gignoux CR, Torgerson DG, et al. Genome-wide association study and admixture mapping identify different asthma-associated loci in Latinos: the genes-environments & admixture in Latino Americans study. *J Allergy Clin Immunol.* 2014; 134:295–305. [PubMed: 24406073]
95. Karunas AS, Iunusbaev BB, Fedorova I, et al. Genome-wide association study of bronchial asthma in the Volga-Ural region of Russia. *Mol Biol (Mosk).* 2011; 45:992–1003. [PubMed: 22295569]
96. Kim SH, Cho BY, Park CS, et al. Alpha-T-catenin (CTNNA3) gene was identified as a risk variant for toluene diisocyanate-induced asthma by genome-wide association analysis. *Clin Exp Allergy.* 2009; 39:203–212. [PubMed: 19187332]
97. Myers RA, Scott NM, Gauderman WJ, et al. Genome-wide interaction studies reveal sex-specific asthma risk alleles. *Hum Mol Genet.* 2014; 23:5251–5259. [PubMed: 24824216]
98. Park HW, Dahlin A, Tse S, et al. Genetic predictors associated with improvement of asthma symptoms in response to inhaled corticosteroids. *J Allergy Clin Immunol.* 2014; 133:664–669. [PubMed: 24486069]
99. Noguchi E, Sakamoto H, Hirota T, et al. Genome-wide association study identifies HLA-DP as a susceptibility gene for pediatric asthma in Asian populations. *PLoS Genet.* 2011; 7:e1002170. [PubMed: 21814517]

100. Smolonska J, Koppelman GH, Wijmenga C, et al. Common genes underlying asthma and COPD? Genome-wide analysis on the Dutch hypothesis. *Eur Respir J*. 2014; 44:860–872. [PubMed: 24993907]
101. Ramasamy A, Kuokkanen M, Vedantam S, et al. Genome-wide association studies of asthma in population-based cohorts confirm known and suggested loci and identify an additional association near HLA. *PLoS ONE*. 2012; 7:e44008. [PubMed: 23028483]
102. Tantisira KG, Lasky-Su J, Harada M, et al. Genomewide association between GLCCI1 and response to glucocorticoid therapy in asthma. *N Engl J Med*. 2011; 365:1173–1183. [PubMed: 21991891]
103. Schauburger EM, Ewart SL, Arshad SH, et al. Identification of ATPAF1 as a novel candidate gene for asthma in children. *J Allergy Clin Immunol*. 2011; 128:753–760. [PubMed: 21696813]
104. Ricci G, Astolfi A, Remondini D, et al. Pooled genome-wide analysis to identify novel risk loci for pediatric allergic asthma. *PLoS ONE*. 2011; 6:e16912. [PubMed: 21359210]
105. Janssen R, Bont L, Siezen CL, et al. Genetic susceptibility to respiratory syncytial virus bronchiolitis is predominantly associated with innate immune genes. *J Infect Dis*. 2007; 196:826–834. The largest candidate gene study of RSV LRI. [PubMed: 17703412]
106. Amanatidou V, Apostolakis S, Spandidos DA. Genetic diversity of the host and severe respiratory syncytial virus-induced lower respiratory tract infection. *Pediatr Infect Dis J*. 2009; 28:135–140. [PubMed: 19106772]
107. Schuurhof A, Bont L, Siezen CL, et al. Interleukin-9 polymorphism in infants with respiratory syncytial virus infection: an opposite effect in boys and girls. *Pediatr Pulmonol*. 2010; 45:608–613. [PubMed: 20503287]
108. Forton JT, Rowlands K, Rockett K, et al. Genetic association study for RSV bronchiolitis in infancy at the 5q31 cytokine cluster. *Thorax*. 2009; 64:345–352. [PubMed: 19131452]
109. Mailaparambil B, Krueger M, Heinze J, et al. Polymorphisms of toll like receptors in the genetics of severe RSV associated diseases. *Dis Markers*. 2008; 25:59–65. [PubMed: 18776592]
110. Puthothu B, Krueger M, Forster J, et al. Interleukin (IL)-18 polymorphism 133C/G is associated with severe respiratory syncytial virus infection. *Pediatr Infect Dis J*. 2007; 26:1094–1098. [PubMed: 18043444]
111. Helminen M, Nuolivirta K, Virta M, et al. IL-10 gene polymorphism at -1082 A/G is associated with severe rhinovirus bronchiolitis in infants. *Pediatr Pulmonol*. 2008; 43:391–395. [PubMed: 18286551]
112. Amanatidou V, Sourvinos G, Apostolakis S, et al. RANTES promoter gene polymorphisms and susceptibility to severe respiratory syncytial virus-induced bronchiolitis. *Pediatr Infect Dis J*. 2008; 27:38–42. [PubMed: 18162936]
113. Thomsen SF, van der Sluis S, Stensballe LG, et al. Exploring the association between severe respiratory syncytial virus infection and asthma: a registry-based twin study. *Am J Respir Crit Care Med*. 2009; 179:1091–1097. [PubMed: 19286626]
114. Kresfelder TL, Janssen R, Bont L, et al. Confirmation of an association between single nucleotide polymorphisms in the VDR gene with respiratory syncytial virus related disease in South African children. *J Med Virol*. 2011; 83:1834–1840. [PubMed: 21837802]
115. Faber TE, Schuurhof A, Vonk A, et al. IL1RL1 gene variants and nasopharyngeal IL1RL1-a levels are associated with severe RSV bronchiolitis: a multicenter cohort study. *PLoS ONE*. 2012; 7:e34364. [PubMed: 22574108]
116. Selvaggi C, Pierangeli A, Fabiani M, et al. Interferon lambda 1–3 expression in infants hospitalized for RSV or HRV associated bronchiolitis. *J Infect*. 2014; 68:467–477. [PubMed: 24389019]
117. Lofgren J, Ramet M, Renko M, et al. Association between surfactant protein A gene locus and severe respiratory syncytial virus infection in infants. *J Infect Dis*. 2002; 185:283–289. [PubMed: 11807709]
118. Lofgren J, Marttila R, Renko M, et al. Toll-like receptor 4 Asp299Gly polymorphism in respiratory syncytial virus epidemics. *Pediatr Pulmonol*. 2010; 45:687–692. [PubMed: 20575099]

119. Stoppelenburg AJ, von Hegedus JH, Huis in't Veld R, et al. Defective control of vitamin D receptor-mediated epithelial STAT1 signalling predisposes to severe respiratory syncytial virus bronchiolitis. *J Pathol.* 2014; 232:57–64. [PubMed: 24105653]
120. Drysdale SB, Prendergast M, Alcazar M, et al. Genetic predisposition of RSV infection-related respiratory morbidity in preterm infants. *Eur J Pediatr.* 2014; 173:905–912. [PubMed: 24487983]
121. Cienciewicki JM, Wang X, Marzec J, et al. A genetic model of differential susceptibility to human respiratory syncytial virus (RSV) infection. *FASEB J.* 2014; 28:1947–1956. [PubMed: 24421397]
122. Randolph AG, Yip WK, Falkenstein-Hagander K, et al. Vitamin D-binding protein haplotype is associated with hospitalization for RSV bronchiolitis. *Clin Exp Allergy.* 2014; 44:231–237. [PubMed: 24447085]
- 123•. Welter D, MacArthur J, Morales J, et al. The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. *Nucleic Acids Res.* 2014; 42:D1001–D1006. This reference for an online GWAS catalog provides information about current GWAS studies. [PubMed: 24316577]
124. Ermers MJ, Janssen R, Onland-Moret NC, et al. IL10 family member genes IL19 and IL20 are associated with recurrent wheeze after respiratory syncytial virus bronchiolitis. *Pediatr Res.* 2011; 70:518–523. [PubMed: 21814157]
125. Wang ZD, Lian D, Shen JL, et al. Association between the interleukin-4, interleukin-13 polymorphisms and asthma: a meta-analysis. *Mol Biol Rep.* 2013; 40:1365–1376. [PubMed: 23070918]
126. Howard TD, Postma DS, Hawkins GA, et al. Fine mapping of an IgE-controlling gene on chromosome 2q: analysis of CTLA4 and CD28. *J Allergy Clin Immunol.* 2002; 110:743–751. [PubMed: 12417883]
127. Barnes KC, Grant AV, Baltadzhieva D, et al. Variants in the gene encoding C3 are associated with asthma and related phenotypes among African Caribbean families. *Genes Immun.* 2006; 7:27–35. [PubMed: 16355111]
128. Hasegawa K, Tamari M, Shao C, et al. Variations in the C3, C3a receptor, and C5 genes affect susceptibility to bronchial asthma. *Hum Genet.* 2004; 115:295–301. [PubMed: 15278436]
129. Inoue H, Mashimo Y, Funamizu M, et al. Association study of the C3 gene with adult and childhood asthma. *J Hum Genet.* 2008; 53:728–738. [PubMed: 18566738]
130. Ermers MJ, Hoebee B, Hodemaekers HM, et al. IL-13 genetic polymorphism identifies children with late wheezing after respiratory syncytial virus infection. *J Allergy Clin Immunol.* 2007; 119(5):1086–1091. [PubMed: 17313976]
- 131•. Manolio TA, Collins FS, Cox NJ, et al. Finding the missing heritability of complex diseases. *Nature.* 2009; 461:747–753. This important genetics article analyzes the potential reasons for why GWAS explain only a small proportion of phenotype variance for a complex trait, despite successes in identifying many genetic variants. [PubMed: 19812666]
132. Thomsen SF, Stensballe LG, Skytthe A, et al. Increased concordance of severe respiratory syncytial virus infection in identical twins. *Pediatrics.* 2008; 121:493–496. [PubMed: 18310197]
133. McCallum GB, Morris PS, Wilson CC, et al. Severity scoring systems: are they internally valid, reliable and predictive of oxygen use in children with acute bronchiolitis? *Pediatr Pulmonol.* 2013; 48:797–803. [PubMed: 22949369]
- 134•. Yang J, Lee SH, Goddard ME, et al. GCTA: a tool for genome-wide complex trait analysis. *Am J Hum Genet.* 2011; 88:76–82. The following starred methodological papers from the Visscher group show how generalized linear-mixed models with genome-wide SNP data can be used to estimate heritability and shared genetic risk in unrelated individuals. [PubMed: 21167468]
135. Falconer, DS.; Mckay, TF. *Introduction to Quantitative Genetics.* 4. Pearson/Prentice Hall; Edinburgh, UK: 2012.
136. Lee SH, Wray NR, Goddard ME, et al. Estimating missing heritability for disease from genome-wide association studies. *Am J Hum Genet.* 2011; 88:294–305. [PubMed: 21376301]
137. Yang J, Manolio TA, Pasquale LR, et al. Genome partitioning of genetic variation for complex traits using common SNPs. *Nat Genet.* 2011; 43:519–525. [PubMed: 21552263]
138. Yang J, Benyamin B, McEvoy BP, et al. Common SNPs explain a large proportion of the heritability for human height. *Nat Genet.* 2010; 42:565–569. [PubMed: 20562875]

139. Lee SH, DeCandia TR, Ripke S, et al. Estimating the proportion of variation in susceptibility to schizophrenia captured by common SNPs. *Nat Genet.* 2012; 44:247–250. [PubMed: 22344220]
140. Lee SH, Yang J, Goddard ME, et al. Estimation of pleiotropy between complex diseases using SNP-derived genomic relationships and restricted maximum likelihood. *Bioinformatics.* 2012; 28(19):2540–2542. [PubMed: 22843982]
141. Jartti T, Gern JE. Rhinovirus-associated wheeze during infancy and asthma development. *Curr Respir Med Rev.* 2011; 7:160–166. [PubMed: 23066381]
142. James KM, Peebles RS Jr, Hartert TV. Response to infections in patients with asthma and atopic disease: an epiphenomenon or reflection of host susceptibility? *J Allergy Clin Immunol.* 2012; 130:343–351. [PubMed: 22846746]
143. Stein RT. Early-life viral bronchiolitis in the causal pathway of childhood asthma: is the evidence there yet? *Am J Respir Crit Care Med.* 2008; 178:1097–1098. [PubMed: 19023035]
144. Kuehni CE, Spycher BD, Silverman M. Causal links between RSV infection and asthma: no clear answers to an old question. *Am J Respir Crit Care Med.* 2009; 179:1079–1080. [PubMed: 19498062]
145. Adamko DJ, Friesen M. Why does respiratory syncytial virus appear to cause asthma? *J Allergy Clin Immunol.* 2012; 130:101–102. [PubMed: 22742838]
146. Gilliland FD, Li YF, Dubeau L, et al. Effects of glutathione S-transferase M1, maternal smoking during pregnancy, and environmental tobacco smoke on asthma and wheezing in children. *Am J Respir Crit Care Med.* 2002; 166:457–463. [PubMed: 12186820]
147. Eder W, Klimecki W, Yu L, et al. Toll-like receptor 2 as a major gene for asthma in children of European farmers. *J Allergy Clin Immunol.* 2004; 113:482–488. [PubMed: 15007351]
148. Bieli C, Eder W, Frei R, et al. A polymorphism in CD14 modifies the effect of farm milk consumption on allergic diseases and CD14 gene expression. *J Allergy Clin Immunol.* 2007; 120:1308–1315. [PubMed: 17919709]
149. Arbour NC, Lorenz E, Schutte BC, et al. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet.* 2000; 25:187–191. [PubMed: 10835634]
150. David GL, Romieu I, Sienra-Monge JJ, et al. Nicotinamide adenine dinucleotide (phosphate) reduced:quinone oxidoreductase and glutathione S-transferase M1 polymorphisms and childhood asthma. *Am J Respir Crit Care Med.* 2003; 168:1199–1204. [PubMed: 12969868]
151. London SJ, Romieu I. Gene by environment interaction in asthma. *Annu Rev Public Health.* 2009; 30:55–80. [PubMed: 18980546]
152. Rava M, Smit LA, Nadif R. Gene-environment interactions in the study of asthma in the postgenomewide association studies era. *Curr Opin Allergy Clin Immunol.* 2015; 15:70–78. [PubMed: 25479314]
153. Ege MJ, Strachan DP, Cookson WO, et al. Gene-environment interaction for childhood asthma and exposure to farming in Central Europe. *J Allergy Clin Immunol.* 2011; 127:138–144. [PubMed: 21211648]
154. Scholtens S, Postma DS, Moffatt MF, et al. Novel childhood asthma genes interact with in utero and early-life tobacco smoke exposure. *J Allergy Clin Immunol.* 2014; 133:885–888. [PubMed: 24315450]
155. Hoffjan S, Nicolae D, Ostrovskaya I, et al. Gene-environment interaction effects on the development of immune responses in the 1st year of life. *Am J Hum Genet.* 2005; 76:696–704. [PubMed: 15726497]
156. Meyers DA, Postma DS, Stine OC, et al. Genome screen for asthma and bronchial hyperresponsiveness: interactions with passive smoke exposure. *J Allergy Clin Immunol.* 2005; 115:1169–1175. [PubMed: 15940130]

EXECUTIVE SUMMARY

Asthma is a complex disease with high heritability & disease risk is likely modified by gene–environment interactions

- Most studies suggest that 60–70% of asthma is heritable.
- Many environmental factors are associated with increased asthma risk making it difficult to study shared genetic risk of asthma and respiratory syncytial virus (RSV) infection.
- Severe infant RSV infection is known to be associated with an increase in risk of asthma.

RSV infection during infancy is known to cause significant morbidity & mortality

- More than half of infants are infected with RSV in the first year of life.
- Approximately, a third of those exposed to RSV have a lower respiratory tract infection (LRI).
- RSV infection is the number 1 reason for hospitalization in infants younger than 6 months of age.
- Infants with RSV LRI have an increased risk of childhood asthma.

Increased asthma risk after infant RSV infection may be due to two nonmutually exclusive factors: a shared genetic predisposition & infant RSV infection acting as a causal agent in asthma development

- Genetic epidemiologic methods can identify common biologic pathways and quantify the extent to which RSV infection severity shares the same genetic predisposition to asthma.

RSV characteristics make it difficult to examine the shared genetic risk with asthma

- RSV circulation is seasonal and dependent on climate or other geographical factors.
- Risk of severe RSV infection is highest in infants between 2 and 4 months of age.
- Because of the seasonal circulation of RSV, infants may not be exposed to RSV during critical periods of development.

Current state of genetics of asthma, RSV infection severity & shared genetic risk

- Over 150 genes have been associated with increased asthma risk, based on genome-wide association studies (GWAS) or candidate gene studies.
- GWAS provides unbiased genome coverage, while candidate gene studies are driven by prior knowledge.

- Only a few RSV infection severity candidate gene studies have been conducted and no GWAS, resulting in minimal overlap with currently identified asthma genes.

Statistical methods based on GWAS data can address important questions about risk for severe RSV infection & the shared genetic risk of severe RSV infection with asthma

- Heritability analysis based on linear mixed models can estimate to what extent severe RSV infection is heritable.
- Genetic correlation analysis, also based on linear-mixed models can determine the shared overlap between RSV infection severity and asthma – even in traits measured in unrelated populations.
- Mediation analysis can examine at a SNP level whether RSV infection severity is on the intermediate pathway between a gene and asthma.
- Gene by environment interactions models can consider the nonadditive relationship between a SNP and asthma risk, when modified by RSV infection severity.

Conclusion

- Severe RSV infection during infancy is a known risk factor for asthma, however, to what extent this association represents a shared predisposition to both diseases and/or is causal, is not known.
- Genetic epidemiologic methods are a time- and cost-efficient means of addressing this difficult, but important question.
- A GWAS of RSV infection severity can provide important insights into the pathophysiological basis of RSV infection and asthma.
- GWAS is currently more affordable than whole genome or exome sequencing and can provide important information about shared mechanisms of disease.

Figure 1. A gene and environmental model of respiratory syncytial virus exposure and asthma (A) All genes or genetic variants associated with severity of response to RSV infection; (B) All genes or genetic variants associated with asthma; and (C) the subset of genes or variants that overlap between RSV illness severity and asthma.
RSV: Respiratory syncytial virus.

Figure 2. Venn diagram showing the relationship between known genes for respiratory syncytial virus infection severity and asthma

Asthma genes refer to multiple asthma phenotypes, including ones identified in sex-interaction analyses. HLA region includes multiple alleles. Intergenic regions that do not map to a gene are excluded, as well as broad linkage regions that suggest the presence of causative loci. Gene names as reported in the papers are presented. Genes separated by commas indicate multiple transcripts.

Figure 3. Casual mediation model

In an unmediated model, (**C**) represents the total effect of one or many SNPs on asthma, estimated from regression. In a mediated model, (**A**) represents the effect of SNPs on RSV illness severity and (**B**) represents the effect of RSV illness severity on asthma, both estimated from regression. (**C'**) is the direct effect of SNP or SNP set on asthma that can be estimated from (**A**) and (**B**) (as indirect effects) and the total effect (**C**).