Exploring the Association between Severe Respiratory Syncytial Virus Infection and Asthma

A Registry-based Twin Study

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***Rationale*: Severe respiratory syncytial virus (RSV) infection is associated with asthma but the nature of this association is imperfectly understood.**

***Objectives*: To examine the nature of the association between severe RSV infection and asthma in a population-based sample of twins.**

***Methods*: Data on hospitalization due to RSV infection was gathered for all twins born in Denmark between 1994 and 2000 (8,280 pairs) and linked to information on asthma obtained from hospital discharge registries and parent-completed questionnaires. Genetic variance components models and direction of causation models were fitted to the observed data.**

***Measurements and Main Results*: RSV hospitalization and asthma were positively associated ($r = 0.43$), and genetic determinants for the two disorders overlapped completely. Modeling the direction of causation between RSV hospitalization and asthma showed that a model in which asthma "causes" RSV hospitalization fitted the data significantly better ($P = 0.39$ for deterioration in model fit) than a model in which RSV hospitalization "causes" asthma ($P < 0.001$ for deterioration in model fit), even when sex, birth weight, and maternal smoking during pregnancy were accounted for.**

***Conclusions*: RSV infection that is severe enough to warrant hospitalization does not cause asthma but is an indicator of the genetic predisposition to asthma.**

Keywords: RSV infection; asthma; twin study; genetic; direction of causation

It is well recognized that infants hospitalized with respiratory syncytial virus (RSV)-associated bronchiolitis are at increased risk of recurrent wheezing and asthma later in childhood (1). The nature of this association is not completely understood. In particular, it is not clear whether the RSV infection plays a direct causative role in asthma or simply identifies infants at risk for subsequent wheezing resulting from an asthmatic predisposition or preexisting abnormal lung function (2). An alternative explanation suggests that RSV-associated bronchiolitis and asthma arise from shared genetic determinants. Several polymorphisms in cytokine genes linked to asthma have been replicated in studies of RSV severity (2). One study reported that an IL-8 gene variant was more frequent in children with wheezing after RSV bronchiolitis compared with children who did not go on to wheeze (3), but an opposite effect of polymorphisms in the IL-8 gene on RSV bronchiolitis and asthma has also been suggested (4).

Using a population-based sample of Danish twins, we recently showed that the susceptibility to severe RSV infection could partly be ascribed to genetic factors (5). In the present study, we explore the nature of the association between severe RSV infection and asthma in the same population of twins. In particular, we aimed to examine the degree of genetic overlap and the direction of causation between these traits.

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Severe respiratory syncytial virus (RSV) infection is associated with asthma development. However, the nature of this relationship is imperfectly understood.

What This Study Adds to the Field

RSV infection that is severe enough to warrant hospitalization does not appear to cause asthma but is an indicator of the genetic predisposition to asthma.

METHODS

Study Population

The study population comprised all live-born twins in Denmark between 1994 and 2000 (6). A total of 8,280 twin pairs were included in the study. Of these, 975 were monozygotic (MZ) twin pairs, 2,427 were dizygotic (DZ) same-sex twin pairs, 3,175 were DZ opposite-sex twin pairs, and 1,703 were twin pairs of unknown zygosity (UZ). The UZ pairs were not included in the present analyses. The ratio of MZ to DZ same sex to DZ opposite-sex twins was 1 to 2.5 to 3.3, and the proportion of males in the population was 51%. Twin zygosity was determined using four questions of similarity and mistaken identity. Zygosity misclassification based on these questions is less than 4% (7). The regional committee on biomedical research ethics approved the protocol (no. KA-20060022).

Measures of RSV Hospitalization and Asthma

Two measures of RSV hospitalization were available. First, information on RSV hospitalization was gathered from the Danish National Patient Registry (8). This registry records all hospitalizations in Denmark since 1977. Children with at least one of the following discharge diagnoses were considered RSV cases: RSV pneumonia (J12.1), RSV bronchitis (J20.5), RSV bronchiolitis (J21.0), and other disease caused

(*Received in original form September 21, 2008; accepted in final form March 11, 2009*)

Supported by grants from the Danish Medical Research Council and the Danish Lung Association (S.F.T.) and by grants from the Dutch Organization for Scientific Research; Social and Behavioral Sciences, NWO/MaGW VIDI-016-065-318 (D.P.) and VENI-016-095-172 (S.v.d.S.).

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Am J Respir Crit Care Med Vol 179. pp 1091–1097, 2009
Originally Published in Press as DOI: 10.1164/rccm.200809-1471OC on March 12, 2009
Internet address: www.atsjournals.org

by RSV (B97.4) (based on the 10th revision of the International Statistical Classification of Diseases and Related Health Problems: ICD-10). We term this measure "discharge diagnosis verified RSV infection." Second, information was available through the RSV database (9). In this database, hospitalized children with verified RSV antigen tested by ELISA or immunofluroscense are recorded. The RSV database covers birth years 1996 to 2003. We term this measure "antigen verified RSV infection." The tetrachoric correlation between discharge diagnosis verified RSV infection and antigen-verified RSV infection was high ($r = 0.93$; *see* RESULTS for more details) to an extent that this caused model fitting problems in subsequent analyses. We therefore decided to collapse these two measures into one RSV measure termed "RSV hospitalization" (i.e., subjects were identified as RSV-cases if they were identified as case by discharge diagnosis verified RSV infection and/or antigen verified RSV infection).

Asthma was also indicated by two measures. First, information on asthma was gathered from the Danish National Patient Registry. According to this registry, an asthma case was defined as a child with at least one of the following discharge diagnoses: allergic asthma (J45.0), nonallergic asthma (J45.1), mixed asthma (J45.8), unspecified asthma (J45.9), or status asthmaticus (J46.9, based on ICD-10). We term this measure "discharge diagnosis asthma." Second, information on asthma was available through the Danish Twin Registry. Information on asthma was collected in 2003 (when the twins were 3–9 years of age) by means of a multidisciplinary postal questionnaire completed by the parents of the twins (response rate, 68%) (6). An affirmative response to the question "Has your child ever had asthma?" identified cases. We term this measure "parent-reported asthma."

Statistical Analysis

The classical twin method can be used to estimate how much of the variance of a trait is explained by genetic (the heritability) and environmental factors. The classical twin method relies on estimating the most likely values of these genetic and environmental variance components given measured trait variances and covariances within the same twin and between the two twins in a pair. Because MZ twins share, besides their early environment and upbringing, all their genes, whereas DZ twins share, besides their early environment and upbringing, only on average half of their segregating genes, any larger covariance (or resemblance) between MZ compared with DZ twins indicates that genetic factors influence the trait variance or the disease liability. The classical twin method can be extended to include several traits simultaneously to estimate whether the same set of genetic (genetic pleiotropy) and environmental factors influence different traits. This analysis uses the information that lies in the measured covariance between different traits. Any larger covariance (or resemblance) between two traits across MZ compared with DZ twins indicates that those two traits share genetic variance. A further extension of the twin method can be used to infer the direction of causation (DOC) between two measured traits. In this instance, the information from the cross-trait cross-twin correlations for a pair of measured traits can be used to resolve the DOC in cross-sectional twin data, particularly if the measured traits have different modes of inheritance. For a further description of the models used in this paper, we refer the reader to Neale and Cardon (10), Gillespie and Martin (11), and Duffy and Martin (12).

First, tetrachoric within-twin and cross-twin correlations were calculated from the raw data. Second, phenotypic factor models were fitted to the observed data (Figure 1), and within-twin and cross-twin correlations were calculated between the factors for asthma and RSV hospitalization (because we used a single collapsed measure of RSV [RSV hospitalization] the factor for RSV is not really a latent factor; see below for more details). Third, a bivariate genetic model was fitted using Cholesky decomposition (Figure 2). In this model, the variance of the asthma and RSV factors was decomposed into variance explained by additive genetic effects (i.e., loci contributing additively to disease risk, A), common environmental effects (i.e., environmental effects that increase the resemblance between family members, C), and unique environmental effects (i.e., environmental effects that are unique to individuals and result in differences between family members, E) (10). From biometrical genetic theory, the expected phenotypic variance is defined as the sum of these three sources of variance, A + C + E. Because MZ twins are genetically identical, the expected covariance for MZ twins equals A + C, whereas the covariance between DZ twins, who share on average 50% of their genes, equals 0.5(A)+ C. Finally, DOC models were fitted (Figure 3). Rather than modeling the relation between asthma and RSV hospitalization through a Cholesky decomposition, we modeled the relation between asthma and RSV hospitalization on the phenotypic level, with one model assuming that RSV hospitalization causes asthma and another model assuming that asthma causes RSV hospitalization. The DOC models are nested under the phenotypic models.

In all models, sex, birth weight (measured in gram and transformed to Z-scores in all subsequent analyses), and maternal smoking during pregnancy (yes/no) were included as covariates on the thresholds. All analyses were performed in Mplus version 4.1 (13). For all models, we used weighted least square estimation using a diagonal weight matrix with standard errors and Satorra–Bentler scaled (mean-adjusted) χ^2 test statistics that use a full-weight matrix (14). Competing hypotheses, represented by different nested models (where the nested model is the more restricted model), can be compared through a weighted χ^2-difference test developed especially for the comparison of the Satorra–Bentler scaled χ^2 values (14). The more restricted model is accepted as the preferred model if its fit is not significantly worse than the fit of the less restrictive model (i.e., if the weighted χ^2 difference test [henceforth referred to as the χ^2_{diff}] is not significant). In this article, we do not report the scaled χ^2 values for each model separately because these values are not informative (i.e., they are not directly interpretable or

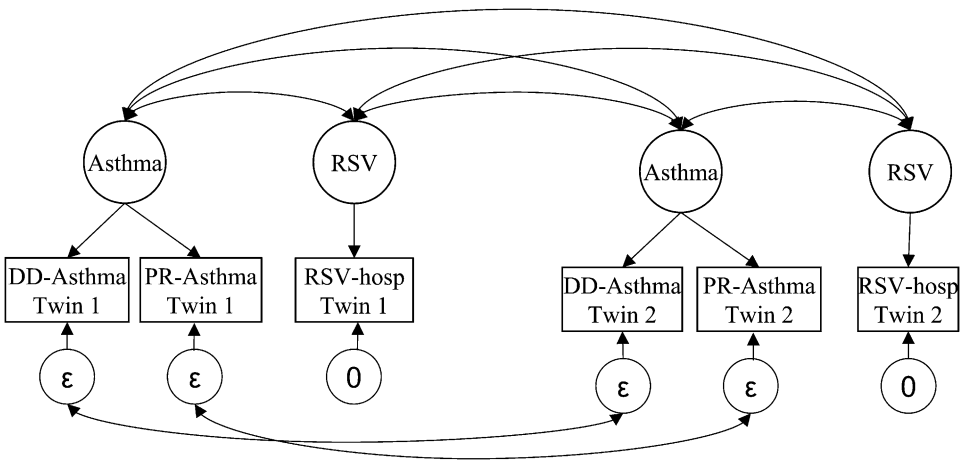

Figure 1. Phenotypic factor model for respiratory syncytial virus (RSV) hospitalization and asthma. *Squares* refer to observed (measured) variables of twin 1 and twin 2; *circles* refer to the latent (unmeasured) variables of twin 1 and twin 2. *Two-headed arrows* denote covariance between factors or variables, and *single-headed arrows* denote directional (regression) relations. The residual variance of the observed variables that is not explained by the latent factors is denoted by ε (or fixed to zero, in the case of RSV-hosp). AV-RSV = antigen verified RSV infection; DD-AST = discharge-diagnosis asthma; DD-RSV = discharge diagnosis–verified RSV infection; PR-AST = parent-reported asthma; RSV-hosp = RSV hospitalization (collapsed measure of AV-RSV and DD-RSV; i.e., either AV-RSV and/or DD-RSV).

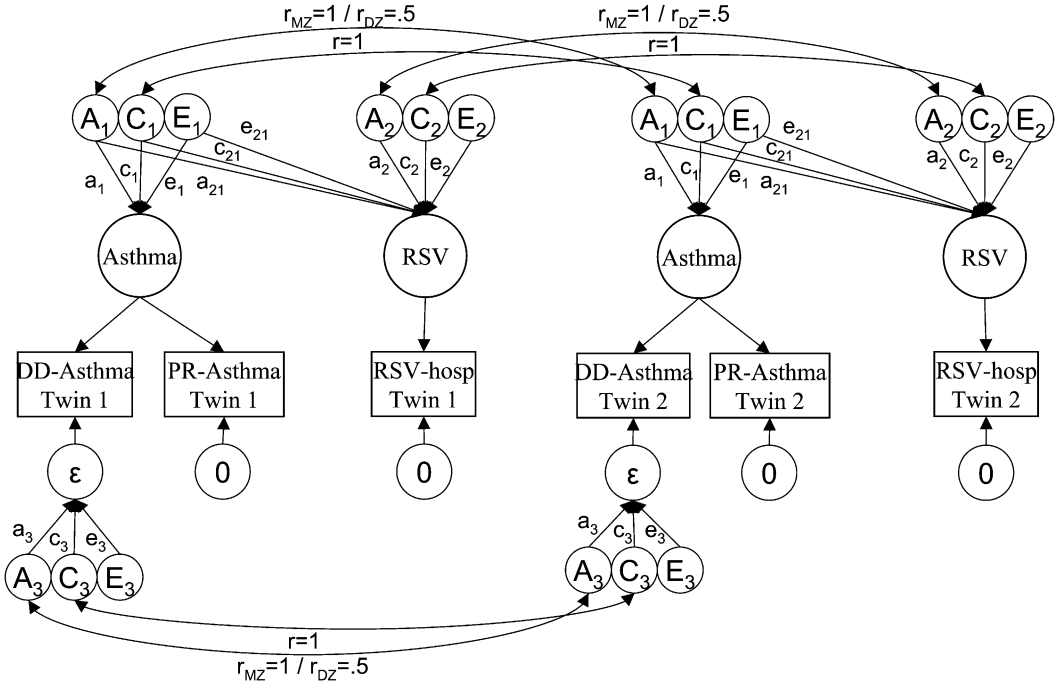

Figure 2. Bivariate Cholesky decomposition of RSV hospitalization and asthma. *Squares* refer to observed (measured) variables of twin 1 and twin 2; *circles* refer to the latent (unmeasured) variables of twin 1 and twin 2. *Two-headed arrows* denote covariance between factors or variables, and *single-headed arrows* denote directional (regression) relations. The residual variance of the observed variables that is not explained by the latent factors is denoted by ε (or fixed to zero, in the case of RSV and parental-reported asthma). A_1, C_1, and E_1 denote additive genetic effects, common environmental effects, and unique environmental effects, respectively, on asthma. A_2, C_2, and E_2 denote these effects on RSV. A_3, C_3, and E_3 denote these effects on the unique variance associated with discharge-diagnosis asthma. In monozygotic (MZ) twins, the A_1 factors, the A_2 factors, and the A_3 factors correlate 1 because these twins are genetically identical. In dizygotic (DZ) twins, these factors correlate 0.5 because these twins share on average 50% of their genes. In MZ and DZ twins, the common environmental factors C_1, C_2, and C_3 correlate 1 because these environmental effects are by definition shared completely between members from the same family. In MZ and DZ twins, the unique environmental factors E_1, E_2, and E_3 correlate 0 because these environmental effects are by definition not shared between members from the same family. The covariance between asthma and RSV is modeled via the paths denoted as a_{21}, c_{21}, and e_{21}, respectively. AV-RSV = antigen-verified RSV infection; DD-AST = discharge-diagnosis asthma; DD-RSV = discharge diagnosis–verified RSV infection; PR-AST = parent-reported asthma; RSV-hosp = RSV hospitalization (collapsed measure of AV-RSV and DD-RSV; i.e., either AV-RSV and/or DD-RSV).

comparable). Rather, we report the weighted χ^2_{diff} tests for the comparison between competing models.

RESULTS

Descriptive Statistics

All 16,560 subjects (8,280 pairs) had complete data on the RSV measures and on discharge diagnosis asthma, whereas only 10,773 subjects (5,154 intact pairs) had complete data on parent-reported asthma. The 5,154 pairs constituted the analytic sample. This missing parent-reported asthma data were due to the fact that not all families returned the questionnaires. This missingness was not random with respect to the RSV measures ($P < 0.001$), age ($P < 0.001$), birth weight ($P < 0.001$), and maternal smoking during pregnancy ($P < 0.001$) but was random with respect to the other measure for asthma (discharge diagnosis asthma), ($P = 0.50$), and sex ($P = 0.42$).

Significant sex effects were observed for parent-reported asthma ($P < 0.001$), discharge diagnosis asthma ($P < 0.001$), discharge diagnosis–verified RSV infection ($P < 0.05$), antigen-verified RSV infection ($P < 0.05$), and the collapsed measure, RSV hospitalization ($P < 0.01$). Male subjects were more often affected than female subjects for all of the observed variables (Table 1).

Among all 1,019 children with a history of RSV hospitalization, 25% had been hospitalized before the age of 3 months, 50% before the age of 6 months, and 75% before the age of 12 months. Ninety-five percent had been hospitalized before the age of 24 months.

Phenotypic Correlations

The phenotypic correlation between the two asthma measures—discharge diagnosis asthma and parent-reported asthma—was high ($r = 0.72$), as was the phenotypic correlation between discharge diagnosis–verified RSV infection and antigen-verified RSV infection ($r = 0.93$) (Table 2). These high phenotypic correlations suggest good reliability of all measures. Furthermore, the collapsed RSV hospitalization measure correlated very highly with the original RSV measures, and correlations of RSV hospitalization with the asthma measures were representative for the correlations that were observed for the two separate RSV measures.

The MZ correlations for asthma were higher (0.81 and 0.95 for discharge diagnosis asthma and parent-reported asthma, respectively) than the DZ correlations (0.51 and 0.62, respectively), suggesting that genetic factors play a role in the individual liability to disease. For the RSV measures, however, MZ and DZ correlations did not differ much (e.g., for RSV hospitalization, MZ and DZ correlations were 0.88 and 0.81, respectively), implying that common environmental effects are much larger than additive genetic effects. The ACE variance decomposition of asthma and RSV hospitalization is therefore expected to differ. Such a difference in variance decomposition is a prerequisite for distinguishing the DOC in DOC models (12). Finally, the cross-trait cross-twin correlations were higher in MZ than in DZ twins, suggesting that asthma and RSV hospitalization share genetic variation.

Phenotypic Models

We fitted a baseline phenotypic model (Figure 1). For both subjects in a twin pair, a latent factor for asthma was specified, which was indicated by the two measures discharge diagnosis asthma and parent-reported asthma. For both measures, residual variances were distinguished, which denote the part of the variance in discharge diagnosis asthma and parent-reported asthma that is not explained by the latent factor. Residual

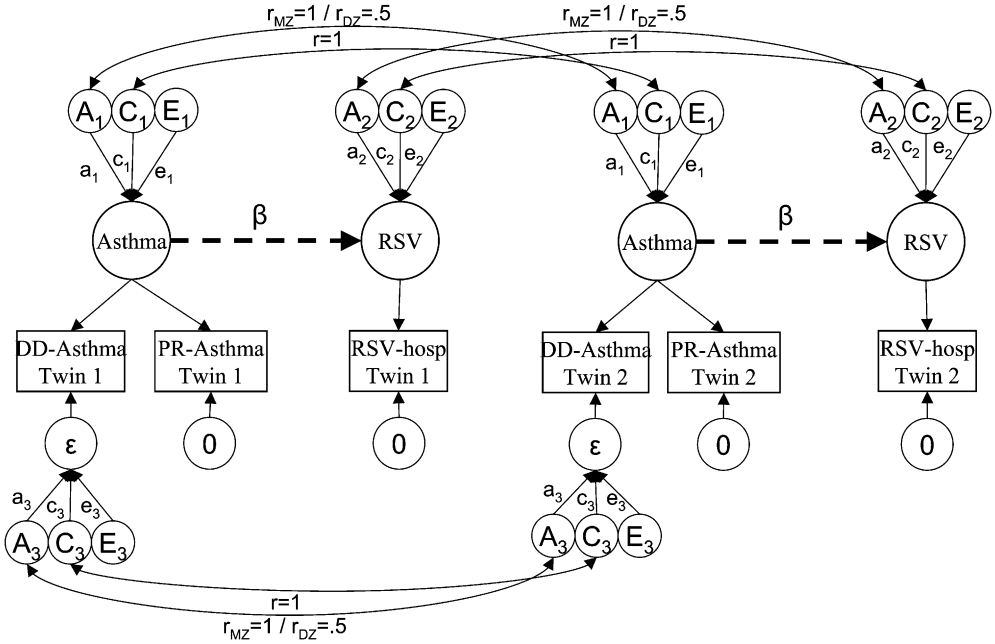

Figure 3. Direction of causation model of RSV hospitalization and asthma. *Squares* refer to observed (measured) variables of twin 1 and twin 2; *circles* refer to the latent (unmeasured) variables of twin 1 and twin 2. *Two-headed arrows* denote covariance between factors or variables, and *single-headed arrows* denote directional (regression) relations. The residual variance of the observed variables that is not explained by the latent factors is denoted by ε (or fixed to zero, in the case of RSV and parental-reported asthma). A_1, C_1, and E_1 denote additive genetic effects, common environmental effects, and unique environmental effects, respectively, on asthma. A_2, C_2, and E_2 denote these effects on RSV. A_3, C_3, and E_3 denote these effects on the unique variance associated with discharge-diagnosis asthma. In MZ twins, the A_1 factors, the A_2 factors, and the A_3 factors correlate 1 because these twins are genetically identical. In DZ twins, these factors correlate 0.5 because these twins share on average 50% of their genes. In MZ and DZ twins, the common environmental factors C_1, C_2, and C_3 correlate 1 because these environmental effects are by definition shared completely between members from the same family. In MZ and DZ twins, the unique environmental factors E_1, E_2, and E_3 correlate 0 because these environmental effects are by definition not shared between members from the same family. The causal effect of asthma on RSV is modeled via the *single-headed dashed arrow* denoted β. (This arrow points from RSV to asthma in the direction of causation model in which RSV causes asthma.) AV-RSV = antigen-verified RSV infection; DD-AST = discharge-diagnosis asthma; DD-RSV = discharge diagnosis–verified RSV infection; PR-AST = parent-reported asthma; RSV-hosp = RSV hospitalization (collapsed measure of AV-RSV and DD-RSV; i.e., either AV-RSV and/or DD-RSV).

variances are calculated rather than estimated when modeling binary data. Because binary data do not have an observed variance, fixing the variance of the factor and the variance of the indicators to 1 scales the structural model, so the residual variance is calculated as 1 – (factor loading^2). The factor for RSV hospitalization had only one indicator (RSV hospitalization), which means that the factor had a one-to-one relation with the indicator, implying a residual variance of zero. The estimation of residual variance is usually desirable because most measures include measurement error and show variation that is not shared with other indicators of the same disease (and is thus not explained by the latent factor). In this case, however, the high phenotypic correlation between the two RSV measures of which RSV hospitalization is composed ($r = 0.93$) and the high MZ correlation for RSV hospitalization ($r = 0.88$) suggest that the RSV hospitalization measure is very reliable and thus that the RSV factor is very reliable.

The factors for asthma and RSV hospitalization were allowed to correlate between twins in a twin pair, as were the residual variances of discharge diagnosis asthma and parent-reported asthma. In this baseline phenotypic model, effects of sex, birth weight, and maternal smoking during pregnancy were modeled on the thresholds. In addition, the variance-covariance structure was allowed to differ for male and female subjects.

The fit of this baseline model was good ($\chi^2[190] = 221.99$; comparative fit index [CFI] = 1.00; root mean square error of approximation [RMSEA] = 0.013). However, discharge-diagnosis asthma was indicated as a Haywood case (i.e., the factor loading was estimated as larger than 1, suggesting that the factor explains more than 100% of the variation in this measure), and for MZ twin pairs, the correlation between the residuals of parent-reported asthma was estimated as larger than 1. Such out-of-bounds parameter estimates can be due to the high correlations between the asthma measures, the information

TABLE 1. CHARACTERISTICS OF A SAMPLE OF 8,280 DANISH TWIN PAIRS, 3 TO 9 YEARS OF AGE

Zygosity	Number (%)*	Males (%)	Females (%)	AV-RSV (%)	DD-RSV (%)	RSV-hosp (%)	PR-AST (%)†	DD-AST (%)
Males	8,491 (51.3)	–	–	371 (4.4)	448 (5.3)	555 (6.5)	698 (12.7)	703 (8.3)
Females	8,069 (48.7)	–	–	325 (4.0)	379 (4.7)	464 (5.8)	457 (8.7)	426 (5.3)
MZ	1,950 (11.8)	992 (50.9)	958 (49.1)	77 (3.9)	104 (5.3)	120 (6.2)	213 (11.8)	159 (8.2)
DZ-ss	4,854 (29.3)	2,574 (53.0)	2,280 (47.0)	191 (3.9)	220 (4.5)	269 (5.5)	499 (11.0)	315 (6.5)
DZ-os	6,350 (38.4)	3,175 (50.0)	3,175 (50.0)	280 (4.4)	317 (5.0)	402 (6.3)	412 (10.3)	449 (7.1)
All DZ	11,204 (67.7)	5,749 (51.3)	5,455 (48.7)	471 (4.2)	537 (4.8)	671 (6.0)	911 (10.7)	764 (6.8)
UZ	3,406 (20.5)	1,750 (51.4)	1,656 (48.6)	148 (4.3)	186 (5.5)	228 (6.7)	31 (6.9)	206 (6.0)
Total	16,560 (100)	–	–	696 (4.2)	827 (5.0)	1,019 (6.2)	1,155 (10.7)	1,129 (6.8)

Definition of abbreviations: AV-RSV = antigen-verified respiratory syncytial virus infection; DD-RSV = discharge diagnosis–verified RSV infection; DZ-os = dizygotic, opposite sex; DZ-ss = dizygotic, same sex; MZ = monozygotic; PR-AST = parent-reported asthma; DD-AST, discharge-diagnosis asthma; RSV-hosp = RSV hospitalization (collapsed measure of AV-RSV and DD-RSV (i.e. either AV-RSV and/or DD-RSV); UZ = unknown zygosity.

* Numbers are individuals.

† For PR-AST, proportions are calculated from available data.

TABLE 2. CORRELATIONS BETWEEN DIFFERENT INDICATORS FOR SEVERE RSV INFECTION AND ASTHMA, IN DANISH TWIN PAIRS, 3 TO 9 YEARS OF AGE

	Phenotypic Correlations (All Twins)					Cross-Trait, Cross-Twin Correlations (MZ Twins)					Cross-Trait, Cross-Twin Correlations (DZ Twins)				
	PR-AST	DD-AST	AV-RSV	DD-RSV	RSV-hosp	PR-AST	DD-AST	AV-RSV	DD-RSV	RSV-hosp	PR-AST	DD-AST	AV-RSV	DD-RSV	RSV-hosp
PR-AST	1					0.95					0.62				
DD-AST	0.72	1				0.57	0.81				0.31	0.51			
AV-RSV	0.34	0.42	1			0.32	0.41	0.77			0.18	0.21	0.76		
DD-RSV	0.32	0.40	0.93	1		0.33	0.48	0.79	0.93		0.17	0.25	0.70	0.84	
RSV-hosp	0.32	0.44	0.99	0.99	1	0.30	0.45	0.78	0.91	0.88	0.17	0.26	0.73	0.81	0.81

Definition of abbreviations: AV-RSV = antigen-verified respiratory syncytial virus infection; DD-RSV = discharge diagnosis–verified RSV infection; DZ-os = dizygotic, opposite sex; DZ-ss = dizygotic, same sex; MZ = monozygotic; PR-AST = parent-reported asthma; DD-AST, discharge-diagnosis asthma; RSV-hosp = RSV hospitalization (collapsed measure of AV-RSV and DD-RSV, i.e., either AV-RSV and/or DD-RSV); UZ = unknown zygosity.

sparsity of measures that are dichotomous in nature, and the limited number of MZ and DZ twin pairs concordant for asthma and/or RSV.

Before attending to these model-fitting problems, we studied the effect of sex, hypothesizing that these problems in the model might disappear if insignificant sex effects were eliminated from the model. Sex effects on the variances appeared nonsignificant and were subsequently dropped from the model (χ^2_{diff} [19] = 23.50; $P = 0.22$), but, as was expected based on the prevalences, sex effects on the thresholds were significant (χ^2_{diff} [3] = 50.07; $P < 0.001$) for all variables. Unfortunately, both model fitting problems remained. The Haywood case was attended to by fixing the factor loading of discharge-diagnosis asthma to 1, which did not result in a significant deterioration of the fit (χ^2_{diff} [3] = 5.14; $P = 0.16$). As a result, the residual of discharge-diagnosis asthma and the correlations between the discharge diagnosis asthma residuals of twin 1 and twin 2 necessarily equal zero. In this restricted model, the MZ correlation between the residuals of parent-reported asthma was still larger than 1. Although fixing this correlation to 1 resulted in a significant deterioration of the model fit (χ^2_{diff} [1] = 6.46; $P = 0.01$), we prefer this model over a model that includes correlations larger than 1. The phenotypic correlation and the twin correlations between the two factors for discharge-diagnosis asthma, parent-reported asthma, and RSV hospitalization are reported in Table 3.

Bivariate Cholesky Models

A bivariate Cholesky model was fitted to the factors for asthma and RSV hospitalization, in which the variance of the factors and the covariance between the factors were decomposed into additive genetic effects (A), common environmental effects (C), and unique environmental effects (E) (Figure 2 and Table 4). The C and E cross-paths from the asthma factor to the RSV factor (i.e., parameters C21 and E21) and the genetic specific of factor RSV (i.e., parameter A2) were not significant, and these parameters could be dropped from the model (χ^2_{diff} [3] = 2.25; $P = 0.52$).

Additive genetic effects explained 79% of the variation observed in asthma but only 14% of the variation observed in RSV hospitalization. Common environmental effects explained only 10% of the variation observed in asthma and 76% of the variation observed in RSV hospitalization. The covariance between asthma and RSV hospitalization was entirely due to shared additive genetic effects. Because the covariance between asthma and RSV hospitalization is genetic and the genetic specific of RSV hospitalization could be dropped from the model, we may also conclude that the genetic correlation between asthma and RSV hospitalization equals 1.

DOC Models

Finally, DOC models were fitted. In these models, the variance of the factors is decomposed into A, C, and E, but rather than modeling a correlation between asthma and RSV, either asthma was modeled to cause RSV hospitalization (Figure 3) or RSV hospitalization was modeled to cause asthma (like Figure 3, but dashed arrow points from RSV hospitalization to asthma). Compared with the fit of the final phenotypic model, modeling asthma to cause RSV hospitalization did not significantly worsen the fit of the model (χ^2_{diff} [2] = 1.86; $P = 0.39$ for deterioration in model fit), whereas modeling RSV hospitalization to cause asthma did result in a significant deterioration of the fit (χ^2_{diff} [2] = 13.91; $P < 0.001$ for deterioration in model fit) (Figure 4). We subsequently fitted direction of causation models in which (*1*) parent-reported asthma was the sole indicator for asthma; (*2*) missing values for parent-reported asthma were imputed with discharge-diagnosis asthma; (*3*) effects of sex, birth weight, and maternal smoking during pregnancy on the thresholds were not controlled for; and (*4*) the model-fitting constraints on the factor loading of discharge-diagnosis asthma and the MZ-correlation between the residuals of parent-reported asthma were elevated. Parameter estimates proved robust against these alternations, and the conclusion with respect to the direction of causation remained unchanged: A model in which asthma causes RSV hospitalization is statisti-

TABLE 3. CORRELATIONS BETWEEN THE FACTORS FOR SEVERE RESPIRATORY SYNCYTIAL VIRUS INFECTION AND ASTHMA, IN DANISH TWIN PAIRS, 3 TO 9 YEARS OF AGE

	Phenotypic Correlation (All Twins)		Cross-Trait, Cross-Twin Correlations (MZ Twins)		Cross-Trait, Cross-Twin Correlations (DZ Twins)	
	RSV	Asthma	RSV	Asthma	RSV	Asthma
RSV	1		0.88 (0.81−0.95)		0.83 (0.80−0.87)	
Asthma	0.43 (0.38−0.49)*	1	0.46 (0.32−0.60)	0.89 (84−0.94)	0.25 (0.18−0.31)	0.50 (0.43−0.56)

Definition of abbreviations: DZ = dizygotic; MZ = monozygotic; RSV = respiratory syncytial virus.
* 95% confidence intervals are reported in parentheses.

TABLE 4. STANDARDIZED PATH COEFFICIENTS FROM THE BIVARIATE CHOLESKY VARIANCE DECOMPOSITION AND THE DIRECTION OF CAUSATION ANALYSIS OF SEVERE RESPIRATORY SYNCYTIAL VIRUS INFECTION AND ASTHMA IN A SAMPLE OF DANISH TWIN PAIRS, 3 TO 9 YEARS OF AGE

	Same-Trait Paths			Cross-Trait Paths			
Model	A	C	E	A21	C21	E21	Regression Paths
Cholesky							
Asthma (factor)	0.89 (0.81−0.98)	0.31 (0.05−0.56)	0.33 (0.26−0.40)				
RSV (factor)	0 (0−0.11)	0.82 (0.37−1)	0.32 (0.20−0.45)	0.37 (0.17−0.58)	0.30 (0−0.83)	0.03 (0−0.28)	
PR-AST	0.44 (0.32−0.57)	0.54 (0.45−0.63)	0 (0−0.01)				
Asthma causes RSV							
Asthma (factor)	0.82 (0.68−0.96)	0.41 (0.16−0.66)	0.40 (0.30−0.50)				
RSV (factor)	0.00 (0−0.22)	0.85 (0.82−0.88)	0.27 (0.18−0.37)				0.45 (0.39−0.51)
RSV causes asthma							
Asthma (factor)	0.80 (0.68−0.92)	0.20 (0−0.59)	0.40 (0.31−0.49)				
RSV (factor)	0.40 (0.20−0.49)	0.86 (0.80−0.92)	0.31 (0.21−0.43)				0.41 (0.35−0.46)

Definition of abbreviations: PR-AST = parent-reported asthma; RSV = respiratory syncytial virus.

cally more plausible than a model in which RSV hospitalization causes asthma.

DISCUSSION

This study showed that RSV infection that was severe enough to warrant hospitalization was significantly associated with asthma. Particularly, comparing the resemblance between MZ and DZ twins suggested a common genetic source for the two disorders. DOC modeling indicated that severe RSV infection was unlikely to be the direct cause of asthma but rather is an indicator of the underlying genetic predisposition to asthma. This result appeared robust against several changes in the fitted model, affirming the reliability of this finding.

These findings expand upon previous studies on the relationship between infant bronchiolitis and childhood asthma. It has been shown that children with asthma develop a more critical manifestation of the RSV infection compared with children without asthma. Also, a number of studies have documented an increased risk of developing severe RSV infection among children with atopic heredity (15), particular for asthma (16), although evidence is circumstantial (17).

Severe RSV infection has been reported to have a long-term detrimental effect on the respiratory system in terms of low lung function, airway hyperresponsiveness, an augmented allergic airway response, and susceptibility to wheezing illness (2). For example, children with RSV bronchiolitis in infancy have an up to 40% risk of developing asthma (17). Moreover, it has been reported that subjects with RSV bronchiolitis in infancy have a significantly reduced lung function later in childhood and as adults (18, 19).

A deficient cytokine response in relation to infection with RSV has been proposed as an indicator of host susceptibility to severe disease (2). Several polymorphisms in cytokine genes have been associated with RSV severity and asthma. In particular, polymorphisms in the IL-4, IL-4RA, IL-10, IL-13, chemokine receptor 5, TGF-β, and TLR-4 genes have been implicated in RSV severity (2, 20), indicating a shared predisposition and/or an interaction between these genetic alterations and the virus.

Childhood asthma likely represents various phenotypes characterized by different genetic and environmental risk factors (21, 22). We did not assess the severity or the age of onset of asthma or objectively verify the diagnosis in terms of impaired lung function and airway responsiveness. Nonetheless, we used several indicators for RSV hospitalization and asthma based on hospital discharge registries, questionnaire, and laboratory data, which increased the validity of our conclusions. Our sample constituted all live-born Danish twins in the period between 1994 and 2000, which limits bias due to selection. Still, nonrespondents to the questionnaire study (subjects with missing data on parent-reported asthma and zygosity) constituted a substantial proportion of the entire population, and these subjects represented a biased selection as reflected in the occurrence of RSV hospitalization, age, birth weight, and maternal smoking but not discharge diagnosis asthma and sex. Also, information on birth weight and maternal smoking were missing for a subset, leaving the analytic sample at 56% of the ascertained population. Furthermore, we found a somewhat higher prevalence of RSV hospitalization in this sample compared with other reports (9). The resemblance between twins for RSV and asthma hospitalization could be influenced by admission of the "unaffected" twin for practical reasons, which could have inflated the prevalence of RSV hospitalization and asthma and attenuated the importance of genetic influences on the disease liability. This may also be the case for the questionnaire-based definition of asthma as parents could be suspected to synchronize responses between the two siblings.

It has been shown that up to 50% of hospitalizations for infant bronchiolitis are due to viruses other than RSV, particularly rhinovirus (RV) and human metapneumovirus (2). RV has been shown to be a major cause of infant bronchiolitis, probably as important as RSV, and coinfection with RSV has been linked to particularly severe illness (2, 23). Moreover, it has been shown that severe infection with RV or human metapneumovirus in infancy has a substantial impact on later asthma risk (24).

We were not able to determine whether the subsequent pattern of asthma associated with a history of severe RSV infection was predominantly intermittent viral respiratory infection–induced asthma or if there was any association with allergen-specific IgE–associated disease. However, we calculated the prevalence of parent-reported hay fever and atopic dermatitis (as proxies of IgE-mediated disease) in patients with asthma with and without a history of severe RSV infection. The prevalence of hay fever was lower in patients with asthma with a history of severe RSV infection compared with patients with asthma without a history of severe RSV infection (4.1 vs. 12.6%), whereas the occurrence of atopic dermatitis was only slightly lower in the first group (21.8 vs. 25.5%). These findings indicate that IgE-mediated mechanisms may play a less important role in the development of asthma in subjects with a history of severe RSV infection compared with subjects without such history.

We speculate that residual confounding could also play a role for the observed association between severe RSV infection and asthma. In particular, socioeconomic status, perinatal exposures, breastfeeding patterns, dietary factors, and exposure to passive smoking during the first years of life have been shown to be important modifiers of the association between severe RSV infection and asthma (25, 26). However, the inclusion of additional confounding factors would have caused more missing subjects and reduced the number of informative families further.

In conclusion, severe RSV infection does not seem to directly cause asthma but rather to be an indicator of the underlying genetic predisposition to asthma. This result should motivate research aimed at elucidating the mechanisms by which severe RSV infection interacts with asthma heredity in the inception of childhood asthma.

Conflict of Interest Statement: S.F.T. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. S.v.d.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. L.G.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. D.P. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. A.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. K.O.K. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. D.L.D. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. V.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. H.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. H.B. has been a consultant and advisor to NeoLab, Nycomed and Merck and paid lecturer for AstraZeneca and Merck. He holds sponsored grants from NeoLab and MedImmune.

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