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Genetic effects of allergen-specific IgE levels on exhaled nitric oxide in schoolchildren with asthma: The STOPPA twin study

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Abstract

Background: Exhaled nitric oxide and blood eosinophils are clinical asthma T-helper type 2 markers in use. Immunoglobulin E (IgE) is often involved in the inflammation associated with atopic asthma. The effect of both blood eosinophils and allergen-specific IgE on exhaled nitric oxide levels is not completely understood. Twin-design studies can improve understanding of the underlying contribution of genetically and/or environmentally driven inflammation markers in asthma. Our aim was to disentangle the covariance between asthma and exhaled nitric oxide into genetic and environmental contributions that can account for inflammation markers in a paediatric population.

Methods: This population-based, cross-sectional twin study enrolled 612 monozygotic (MZ) and same-sex dizygotic (DZ) schoolchildren. Multivariate structural equation modelling was utilized to separate the covariance between asthma and exhaled nitric oxide into genetic and/or environmental effects, taking allergen-specific IgE level and blood eosinophil count into account while controlling for confounding factors.

Results: The cross-twin/cross-trait correlations had a higher magnitude in the MZ twins than in the DZ twins, indicating that genes affect the association. The likelihood ratio test for model fitting resulted in the AE model (ie additive genetic effects, A, and non-shared environmental effects, E) as the most parsimonious. A majority, 73%, of the phenotypic correlation between asthma and exhaled nitric oxide, $r = .19$ (0.05-0.33), was attributable to genetic effects which mainly was due to the allergen-specific IgE level.

Conclusions: This study indicates that the association between asthma and exhaled nitric oxide in children is to a large extent explained by genetics via allergen-specific IgE level and not blood eosinophils. This might partly explain the clinical heterogeneity in this group. A next step could be to include allergen-specific IgE level in multivariate omic studies.

KEYWORDS
eosinophils, immunoglobulin E, inflammation, twins

1 | INTRODUCTION

Asthma is the most common chronic disease in children,[1] and it is defined as an obstructive and often underlying airway inflammatory disease.[2] T-helper type 2 (T2) responses are thought to be central mediators of inflammation in asthma,[3] and a widely used clinical marker to measure possible T2 inflammation is exhaled nitric oxide (FE_{NO}).[3,4]

FE_{NO} is easy to measure in exhaled breath, and its level corresponds directly with asthma and inflammation in the bronchial epithelium.[5] FE_{NO} can therefore aid asthma diagnosis, and, if correctly applied and interpreted, identify patients at risk of exacerbation.[6] In clinical practice, generalized cut-off values of FE_{NO} have so far been difficult to translate to individual patients due to unknown contribution of factors that influence the FE_{NO} value, such as allergen-specific immunoglobulin E level (IgE), blood eosinophil counts,[7] tobacco smoke exposure,[8] upper airway infection, age and height.[9]

The interest of measuring the number of blood eosinophils has increased in recent years since the introduction of anti-interleukin-5 therapy for severe eosinophil asthma.[10] High blood eosinophils appear to be related to poor asthma control, hospitalisation[11] and reduced lung function development in adults.[12] Hence, both FE_{NO} and blood eosinophil count reflect ongoing T2 inflammation. However, the inflammatory contribution of blood eosinophil counts on the association between asthma and FE_{NO} is debated and not completely understood.[11] FE_{NO} levels are activated by interleukin-4/-13 and blood eosinophil count by interleukin-5.[13] However, these markers may play a very different role in adults and children due to differences in underlying pathophysiology.[14] Nonallergic eosinophilic asthma, not mediated by specific IgE, develops later in life and is rarely present in childhood,[15] indicating different mechanisms involved in regulating FE_{NO} and eosinophils in children and adults. IgE is an important clinical biomarker, which is often involved in atopic asthma, the most common form of asthma in children.[16] The airway inflammation involved in atopic asthma is recognized with both increased total IgE concentration and an elevated FE_{NO} fraction, as well as activation of eosinophilic granulocytes.[16,17] In sensitized children, blood eosinophil count has been shown to be associated with increased level of FE_{NO}.[18] Still, the relative contribution of allergen-specific IgE level and blood eosinophil count on FE_{NO} in children with asthma is not established.

Twin studies provide a unique method for determining the contribution of genetic and environmental sources of variation in a disease or a phenotype.[19] The multivariate twin design can aid in the estimation of the same genetic and/or environmental factors that influence different diseases and intermediate phenotypes.[20] This can broaden our understanding of asthma biomarkers and inform gene-mapping efforts.[21] We have previously shown that the association between asthma and FE_{NO} was mainly explained by genetics and allergic sensitization.[22] Therefore, our goal here was to further disentangle the association between asthma and FE_{NO}, by estimating the relative contribution of genetic and/or environmental effects from both allergen-specific IgE level and blood eosinophils. These potential shared genetic origins and environmental contributions will be studied in a multivariate twin study, thereby avoiding inflated type 1 error by multiple testing.

Key Message

Allergen-specific IgE level impacts the association between asthma and FE_{NO} by a genetic component. This may give us a hint of including IgE diagnostics when treating and managing asthma according to FE_{NO} levels in the future.

2 | METHODS

2.1 | Study design and study sample

The Swedish Twin Study on Prediction and Prevention of Asthma (STOPPA) is a population-based, cross-sectional twin study on childhood asthma in discordant, concordant and healthy concordant twins.[23] The study participants were recruited from the Child and Adolescent Twin Study in Sweden (CATSS),[24] a study initiated in 2004 that included all twins born from July 1992 and onwards, identified through the Swedish Twin Registry.[25] Validated questions on asthma ever (yes/no) 'Does he/she have, or has he/she had asthma?'[24,26] and wheezing (current or after three years of age) according to the International Study of Asthma and Allergies in Childhood (ISAAC) were used to identify 9- to 14-year-old twins discordant and concordant for asthma or wheeze and healthy control twins.[26] Monozygotic (MZ) and same-sex dizygotic (DZ) twin pairs who were raised together were recruited to STOPPA from all parts of Sweden and distributed throughout the whole year. Participants were invited to a clinical examination including questionnaires and objective measures at eight different study sites.[23] The study population (n = 752) was classified as asthma concordant (31%), asthma discordant (38%) and healthy concordant (31%), according to the recruitment algorithm of asthma status, as described in detail elsewhere.[23] One twin pair had unknown zygosity and 53 did not consent to blood sampling. The final analytic sample consisted of 612 individuals (81.4% of the study population with full information on exposure and all covariates). The study was approved by

the Regional Ethical review board in Stockholm, Sweden. Informed consent was obtained from all the participants and their parents.

2.2 | Variables

2.2.1 | Asthma

All twins and their parents completed questionnaires. The parental questionnaire, which has previously been validated against health care registers with good agreement,[27] included questions on the parent's lifestyle, background and medical history, followed by a section on each twin's general health status, lifestyle and medical history including current asthma defined as reporting positively to the question 'Does your child have asthma?'.[23]

2.2.2 | FE_{NO}

Exhaled nitric oxide was measured during an exhalation of at least 6 seconds at a flow of 50 mL/s (FE_{NO}), measured with a hand-held electrochemical analyzer (NIOX Mino, Aerocrine) according to the guidelines.[28] The average integer value of FE_{NO} (parts per billion) was recorded based on two consecutive measurements if they differed by <5% or based on three measurements if they differed by >5%.

2.2.3 | Sensitization to airborne allergens

More than 90% of the participants underwent blood sampling, and serum was analysed for IgE antibodies to Phadiatop®, Thermo Fisher Scientific, a screening test for sensitization to a mix of common inhalant allergens (birch, timothy, mugwort, cat, dog, horse, house dust mites [*Dermatophagoides pteronyssinus* and *farina*] and mould [*Cladosporium herbarum*]). Sensitization was regarded as a level of ≥0.35 kU_A/L corresponding to a fluorescence intensity of 168 response units, and this defined the categorical (1/0) *IgE-positive* variable. The numeric IgE level to Phadiatop®, here termed the *allergen-specific IgE level*, was used as a continuous IgE variable and has been described previously.[29] IgE values below the level of quantification of 0.1 kU_A/L were assigned a value 0.09 kU_A/L, and values above 100 kU_A/L were set at 100 kU_A/L, as described elsewhere.[30] All samples were analysed at the Department of Clinical Immunology and Transfusion Medicine at the Karolinska University Hospital Solna, Sweden.

2.2.4 | Eosinophils

Samples of venous blood were collected in 4-mL EDTA tubes and transported to the local chemistry laboratory for analysis of white blood cell count including blood eosinophils (1×10^9 counts/L) according to standard operating procedure at each specific site.

2.2.5 | Inhaled corticosteroids

Parents confirmed inhaled corticosteroid (ICS) treatment by answering yes to the questions 'Does he/she have, or has he/she had asthma?'[24] and 'Has your child used ICS treatment regularly during the last 12 months?'

2.2.6 | Zygosity

Data on zygosity were retrieved from the CATSS study. A majority of the twins had their zygosity determined by DNA analysis (84.3%), with the remaining assessed via an algorithm of five questions on twin similarity, a validated technique to determine zygosity with at least 95% accuracy.[24]

2.2.7 | Age

Information on age was collected from the questionnaire and is included as a covariate in the analyses. Age when samples were taken was calculated as date of measurement minus date of birth.

2.2.8 | Socioeconomic status (SES)

As a proxy for SES, we used the parental (maternal or paternal) highest education retrieved from the questionnaire.

2.2.9 | Any parental asthma

To assess the parental history of asthma, we collected the following item from the questionnaires: 'Does the mother/father have asthma?' and created a new variable 'any parental asthma', based on whether either the mother or father or both had asthma.

2.2.10 | Parental current smoking

Smoking was assessed from the questionnaire with the following question: 'Does the mum/dad smoke?'

2.3 | Statistical analyses

We analysed asthma, FE_{NO}, allergen-specific IgE level and eosinophils, in a four-variate twin model. In this model, the covariance between variables within individuals, as well as the covariance between twins in pairs, is explicitly modelled in structural equation models. Asthma was analyzed as a binary variable and adjusted for age and sex. The data for FE_{NO}, allergen-specific IgE level and eosinophils were all log-transformed to obtain a distribution closer to

normal, and adjustments were done for ICS, age and sex. Due to the sampling scheme, the population may have a skewed distribution (compared to the source population) of investigated variables; thus, we re-weighted all analyses according to sampling probability.

2.4 | Assumptions testing

First, a saturated model was fitted, which included separate estimates for means, prevalence rates, variances, and covariances between 'twin 1' and 'twin 2', according to random assignment for order in pairs and separately between MZ and DZ twin pairs. We then proceeded to fit a model where we assumed symmetry within each zygosity; that is, the twin order was not associated with means/prevalences and variances or with within-individual covariance between the traits, named 'Symmetric'. Finally, a model where means and variances were additionally assumed to be the same across zygosities was fitted corresponding to the basic assumptions needed for a quantitative genetic model, named 'Assumption'.

2.5 | Observed correlations

We presented correlations from the 'Symmetric' model described above. The phenotypic correlations, r_{ph}, were based on the within-twin/cross-trait correlations. Intra-class correlations (ICC) are the correlations between the same variable measured in one twin and in his/her co-twin (ie cross-twin/within-trait). If the absolute value of ICC is larger in MZ twins than in DZ twins, this indicates that genes are involved in the association. The cross-twin/cross-trait (CTCT) correlations represent the correlations between one variable in one twin and another in the co-twin. If the absolute value of the CTCT is larger in MZ twins than in DZ twins, this implies that genes influencing both traits (at least partly) overlap. Pearson correlations were calculated for the associations between continuous traits, while tetrachoric for binary (asthma) and biserial correlations were calculated for both binary and continuous traits.

2.6 | Quantitative genetic model

Based on the differences in genetic similarity for twins with different zygosities, MZ twins have a correlation of 1 for additive genetic effects (A), representing the combined effect of alleles at a locus and across loci that add up, whereas DZ twins have a correlation of .5[31] Dominance genetic effects (D or d^2) also contribute to twin pair similarity and the index interaction of gene alleles at the same locus (dominance), and are assumed correlated 1 between MZ twins and 0.25 between DZ twins. Furthermore, both types of twins are assumed to have an equal correlation of 1 for environmental effects that both twins share (C or c^2), such as perinatal and home environment, whereas unique environmental effects that twins in pairs do not share (E or e^2), such as accidents, are modelled with a correlation of 0 between twins. Thus, a higher correlation in MZ twins than in DZ twins would represent the effect of the higher proportion of genes shared among MZ twins.[32]

The multivariate genetic model estimates the genetic and environmental contributions to the phenotypic correlation between asthma and FE_{NO} and the degree that can be accounted for by sensitization to aeroallergens and blood eosinophils. The phenotypic correlations were decomposed into combinations of A, D, C and E, depending on which model was fitted. We fitted a series of structural equation models estimating the maximum-likelihood genetic and environmental variance components of variables, and the covariance between these. We performed likelihood ratio tests to find the best-fitting model.

We were interested in the correlation between asthma and FE_{NO} and what potentially could affect this correlation in terms of genetic and environmental influences from other factors. Therefore, we proceeded to fit a four-variate 'Cholesky model' to disentangle the sources of variance and covariance into genes and environment. In a Cholesky model, the order the variables appear is important; the 'left' variables influence the variables to the 'right', but not vice versa[33] (see Figure 1) to allow estimation of the influence (ie genetic and/or environmental) of allergen-specific IgE level and eosinophils on the association between asthma and FE_{NO}. Since we were interested if allergen-specific IgE level and eosinophils could influence asthma and FE_{NO}, we decided to model the variables in this order.

We fitted an ACE model, that is a model with A, C and E sources of variance and covariance. We also fitted an ADE model, AE model and CE model. We tested whether the nested models had poorer fits to the data using likelihood ratio tests. We also used the Akaike Information Criterion (AIC) to assess the model fit. The AIC favours model parsimony and allows for comparisons across non-nested models. In addition, we compared other models with the ACE model (base) to assess model fit.

Figure 1 shows the Cholesky model (here, the AE model is taken as an example) with the observed variables (eosinophils, allergen-specific IgE level, asthma and FE_{NO}) in relation to the unobserved latent factors, which constitute of additive genetic effects and unique/unshared environmental effects ($A_{1\text{-}4}$ and $E_{1\text{-}4}$, respectively), here represented by circles, which are connected by the paths $a_{11\text{-}44}$ and $e_{11\text{-}44}$. Thus, the variance in, and covariance between, asthma and FE_{NO} may be explained by the variance in eosinophils and allergen-specific IgE level, but not vice versa. Analyses were performed using the statistical software R,[34] version 3.6.1, and the package OpenMx,[33] version 2.15.5.

Additional details on calculated contributions to the correlations between asthma and FE_{NO} are provided in the Supporting Information.

2.7 | Sensitivity analyses

In addition, to instigate the potential effect of the season, when the samples were taken, in sensitivity analyses we additionally adjusted for season at sampling time.

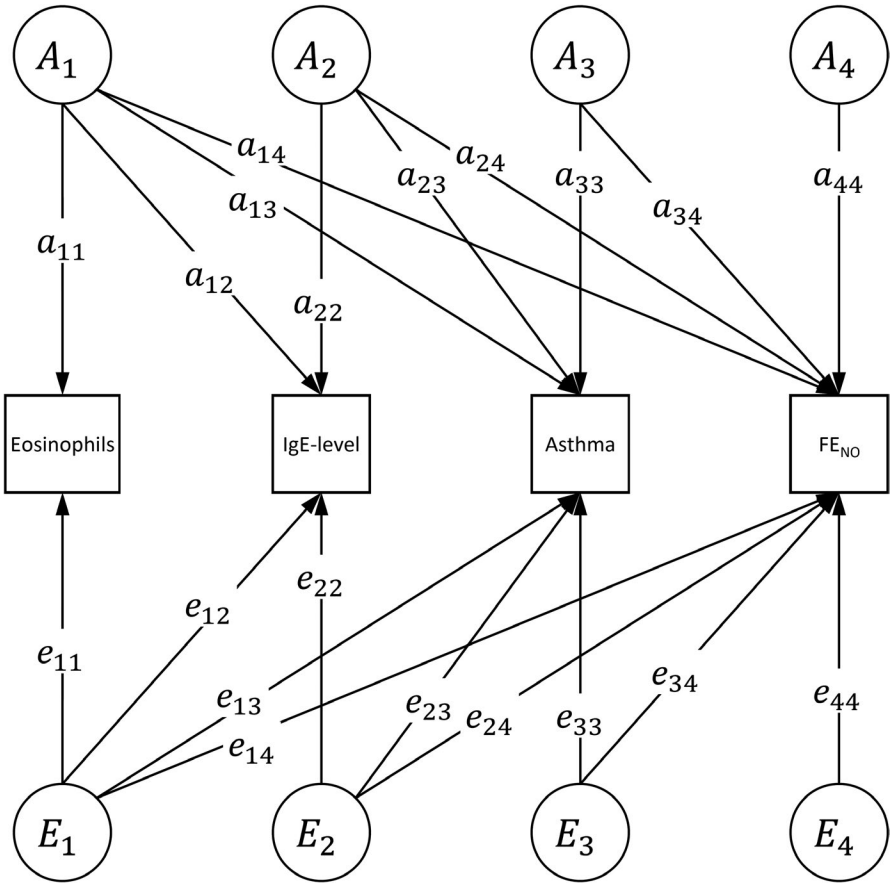

FIGURE 1 Cholesky AE model. Path diagram of association within one individual. Capital A and E refer to latent factors, lower-case a and e refer to path coefficients onto the observed variables. Observed variables are depicted with a square. IgE level = continuous value of allergen-specific IgE level. Note Variances of latent factors not depicted, but assumed fixed at 1

3 | RESULTS

Table 1 gives an overview of the study population which had a mean age of about 12.5 years in both groups and with 58% males in the current asthma group. The percentage of any parental asthma was higher in the asthma group (46%) than in the group reported no current asthma (16%). The geometric means of FE_{NO} and the allergen-specific IgE level were higher (18 and 2.3, respectively) in the asthma group than in the no current asthma group (12.9 and 0.3, respectively). The mean blood eosinophil count was higher in the asthma group (0.4 vs 0.3), but the reported ICS was lower in the no current asthma group, at 0.2%, than in the asthma group, at 35%.

Here, we will only present results from models using the continuous variable allergen-specific IgE level to maximize statistical power. The Supporting Information shows results for the dichotomous variable IgE positive.

3.1 | Observed correlations

The 'Symmetric' model provides estimates of correlations, as estimated for MZ and DZ independently, and as all twins together (Table 2).

Table 2 present observed maximum-likelihood correlations. Most r_{ph} correlations were statistically significantly different from 0, and about the same magnitude in both the MZ and DZ twins (except for the correlation between eosinophils and asthma which was .29 and significant in MZ twins but was −.05 and non-significant in DZ twins), indicating that all variables are associated within individuals. All ICCs were statistically significantly different from 0 and higher in the MZ twins than in the DZ twins, indicating a heritable component for the univariate measures. All CTCTs had a higher magnitude in the MZ twins than in the DZ twins indicating that genes affect the association between all variables.

3.2 | Model fitting

The likelihood ratio test was first compared with the saturated model (Table S2A) and then with the ACE model (Table S2B, with best likelihood among quantitative genetic models). A statistically non-significant drop in fit ($P = .884$) was observed when comparing the AE model against the ACE model, making the AE model the most parsimonious/final model (Table S2B).

3.3 | Quantitative genetic model

Figure 2 and Table 3 present the correlation between asthma and FE_{NO} separated into genetic and environmental sources. These were factors

TABLE 1 Descriptive statistics of the study individuals

	Current asthma[a], n = 127		No current asthma, n = 465		
	Mean (SD)	n (%)	Mean (SD)	n (%)	*P*-value
Age (y)	12.3 (1.5)	127 (100)	12.6 (1.4)	465 (100)	.04
Age at sampling-time (y)	12.3 (1.6)	127 (100)	12.6 (1.4)	465 (100)	.04
Sex					
Male		73 (57.5)		249 (53.5)	.43
Female		54 (42.5)		216 (46.5)	
Parental education					
<9 y completed		0		0 (0)	.16
9-12 y completed		34 (26.8)		105 (22.6)	
>12 y completed		93 (73.2)		360 (77.4)	
Any parental asthma[b]					
Yes		58 (45.7)		72 (15.5)	<.001
No		66 (52.0)		374 (80.4)	
Missing		3 (2.4)		19 (4.1)	
Smoking mother, current					
Yes		10 (7.9)		54 (11.6)	.14
No		70 (55.1)		220 (47.3)	
Missing		47 (37.0)		191 (41.1)	
Smoking father, current					
Yes		13 (10.2)		35 (7.5)	.36
No		68 (53.5)		252 (54.2)	
Missing		46 (36.2)		178 (38.3)	
FE_{NO}					
≥18.25 ppb		55 (43.3)		92 (19.8)	<.001
Arithmetic mean	25.5 (22.1)	122 (96.1)	15.8 (13.0)	436 (93.8)	<.001
Geometric mean	18.0	122 (96.1)	12.9	436 (93.8)	<.001
Allergen-specific IgE level, kU_A/L					
Arithmetic mean	19.6 (26.3)	115 (90.6)	7.0 (17.9)	433 (93.1)	<.001
Geometric mean	2.3	115 (90.6)	0.3	433 (93.1)	<.001
IgE positive[c]					
No		40 (31.5)		283 (60.9)	<.001
Yes		75 (59.1)		150 (32.3)	
Eosinophil cell count, 1×10^9 counts/L					
Arithmetic mean	0.4 (0.4)	115 (90.6)	0.3 (0.3)	422 (90.8)	<.001
Geometric mean	0.3	115 (90.6)	0.2	421 (90.5)	<.001
ICS regularly[d]					
No		83 (65.4)		464 (99.8)	<.001
Yes		44 (34.6)		1 (0.2)	
Zygosity					
MZ		72 (56.7)		205 (44.1)	.01
DZ		55 (43.3)		260 (55.9)	
Season at sampling-time[e]					
Spring (March-April-May)		38 (29.9)		105 (22.6)	
Summer (June-July-Aug)		26 (20.5)		60 (12.9)	.004
Autumn (Sept-Oct-Nov)		53 (41.7)		214 (46.0)	
Winter (Dec-Jan-Feb)		10 (7.9)		86 (18.5)	

Abbreviations: n, number of participants; ppb, parts per billion; SD, standard deviation.

[a]Asthma is derived from questionnaire by parents answering the question: 'Does your child have asthma?' (n = 20 had missing value on 'current asthma' but the method allows missing values in the outcome variables).

[b]Based on whether either the mother or father answered 'yes' if they had asthma on the questionnaire.

[c]Sensitization to aeroallergens: sIgE ≥ 0.35 kU_A/L to Phadiatop (birch, timothy, mugwort, cat, dog, horse, house dust mites and mould).

[d]Regular use of ICS during the last 12 mo.

[e]There were no within-twin pair variability regarding season at sampling time.

TABLE 2 Twin correlations

Correlation	MZ twins Estimate (95% CI)	DZ twins Estimate (95% CI)	All twins Estimate (95% CI)
r_{ph} eosinophils and IgE level[a]	**0.26 (0.12-0.39)**	**0.24 (0.12-0.35)**	**0.24 (0.16-0.33)**
r_{ph} eosinophils and asthma[b]	**0.29 (0.07-0.51)**	−0.05 (−0.27 to 0.18)	0.11 (−0.06 to 0.27)
r_{ph} eosinophils and FE_{NO}[a]	**0.34 (0.20-0.47)**	**0.28 (0.17-0.39)**	**0.30 (0.22-0.39)**
r_{ph} IgE level and asthma[b]	**0.33 (0.13-0.52)**	**0.29 (0.11-0.47)**	**0.30 (0.16-0.44)**
r_{ph} IgE level and FE_{NO}[a]	**0.45 (0.34-0.57)**	**0.40 (0.30-0.50)**	**0.42 (0.35-0.50)**
r_{ph} Asthma and FE_{NO}[b]	**0.21 (0.00-0.42)**	0.17 (−0.02 to 0.35)	**0.18 (0.04-0.33)**
ICC asthma[c]	**0.83 (0.66-1.00)**	**0.48 (0.14-0.83)**	NA
ICC FE_{NO}[a]	**0.67 (0.56-0.77)**	**0.34 (0.20-0.48)**	NA
ICC IgE level[a]	**0.63 (0.55-0.72)**	**0.26 (0.11-0.41)**	NA
ICC eosinophils[a]	**0.56 (0.43-0.69)**	**0.30 (0.14-0.46)**	NA
CTCT eosinophils and IgE level[a]	**0.18 (0.04-0.32)**	0.02 (−0.11 to 0.14)	NA
CTCT eosinophils and asthma[b]	**0.28 (0.06-0.50)**	0.00 (−0.22 to 0.21)	NA
CTCT eosinophils and FE_{NO}[a]	**0.17 (0.02-0.32)**	**0.12 (0.00-0.24)**	NA
CTCT IgE level and asthma[b]	**0.29 (0.10-0.47)**	0.09 (−0.11 to 0.29)	NA
CTCT IgE level and FE_{NO}[a]	**0.32 (0.20-0.45)**	**0.14 (0.02-0.26)**	NA
CTCT Asthma and FE_{NO}[b]	0.17 (−0.04 to 0.39)	−0.08 (−0.28 to 0.12)	NA

Note: Bold = statistically significantly different from zero. r_{ph} = phenotypic correlation, that is the correlation between variables within individuals, within-twin/cross-trait. ICC = intra-class correlation, that is the correlation between the same variable measured in one twin and in her co-twin, cross-twin/within-trait. CTCT = cross-twin/cross-trait correlation, that is the correlation between one variable in one twin and another in the co-twin, IgE level = continuous value of allergen-specific IgE level. Correlations adjusted for covariates sex, age at examination and ICS-medication (asthma not adjusted for ICS).

[a]Pearson correlation.

[b]Biserial correlation.

[c]Tetrachoric correlation.

uniquely related to allergen-specific IgE level, factors unique to eosinophils, factors shared by allergen-specific IgE level and eosinophils, and factors shared between asthma and FE_{NO}, as estimated in the (best-fitting) AE model (Supporting Information method outlines how the separation into unique and shared sources has been achieved).

In the best-fitting AE model, a significant phenotypic correlation, r_{ph}, was noted between asthma and FE_{NO} (r_{ph} = .19; Table 3). The part of the phenotypic correlation, which can be attributable to additive genetic effects, $r_{ph\text{-}a}$, due to allergen-specific IgE level was statistically significant ($r_{ph\text{-}a}$ = .10) and accounted for half (54%) of the correlation between asthma and FE_{NO}. All other estimates were non-significant.

Other quantitative genetic models (ACE, ADE and CE), the path coefficients from the AE model, as well as the heritability estimates, are shown in Table S3, Table S4A,B.

Twin correlations and results from the quantitative genetic models obtained when the categorical variable IgE positive was used instead of allergen-specific IgE level can be found in the Tables S5, S6. Overall, the results using the categorical IgE positive were very similar to the results obtained from the continuous allergen-specific IgE level.

3.4 | Sensitivity analyses

Results from the sensitivity analyses when season at sampling-time was added as a covariate can be found in the Tables S7-S11B and Figure S2. Overall, the results were very similar and no differences from the original analyses were found.

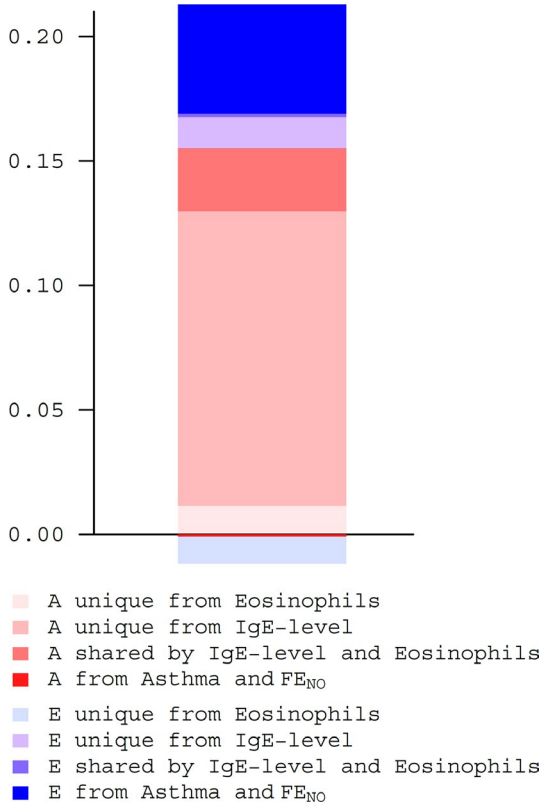

FIGURE 2 Explained phenotypic correlation between asthma and FE_{NO}, re-weighted by sampling probability and adjusted for ICS use. The height of the bar represents the phenotypic correlation, r_{ph}, which is further decomposed into genetic and environmental parts explained by allergen-specific IgE levels, eosinophils, asthma and FE_{NO}. The colour coding represents additive genetic effects, factor A (reddish) or unique environmental effects, factor E (blueish), on the total r_{ph}. IgE level = continuous value of allergen-specific IgE level

4 | DISCUSSION

In this population-based twin study, we disentangled the genetic and environmental sources of covariation between asthma and FE_{NO} by analysing the effect of blood eosinophils and allergen-specific IgE level. More than half (54%) in the total covariance between FE_{NO} and asthma was due to genetically driven effects of the IgE level to airborne allergens. Thus, our results indicate that genetically driven allergen-specific IgE level, but not blood eosinophil counts, is part of the same underlying construct that creates significant correlation between asthma and FE_{NO} in schoolchildren.

This study provides further understanding of genetic influence from the allergen-specific IgE level on FE_{NO} in children with asthma. Complex genetic inheritance in asthma susceptibility has been reported, where more than one hundred genetic variants have been implicated.[35] Although just a subset of these genetic variants has been replicated, the genetic contribution to the asthma and FE_{NO} association seems unclear.[22] Genetic studies have found a link between FE_{NO} values and a few genetic loci[36,37] and the genetics of the IL-4/IL-13 pathway have been linked to IgE levels of childhood asthma.[38,39] Interestingly, Thomsen et al studied the covariance between FE_{NO} and total IgE and found that 93% of the phenotypic correlation could be explained by genetic factors.[40] Thus, previous results point to the fact that genetics play an important role in the allergic asthma phenotype. Here, we show that allergen-specific IgE level, but not blood eosinophils, highly impacts the asthma FE_{NO} association by a genetic component. This may give us a hint of including IgE diagnostics when treating and managing childhood asthma according to FE_{NO} levels in the future.

Previous twin research on asthma phenotypes has applied bivariate modelling to their data,[21,40-43] thereby separating the covariance of genetic and environmental determinants from two sources. Results within these bivariate studies point to a large extent of a common genetic background between asthma phenotypes.[40-43] Here, we include a multivariate modelling with four different sources to investigate whether they share common genetic and/or environmental origins. The advantage of using a multivariate over a bivariate model is that the relationships between several variables can be found simultaneously. Allergen-specific IgE level can be considered a cause of allergic asthma as it is involved early in the inflammatory process, which also implicates an increase in eosinophils.[44] Here, we found significant genetic influence from allergen-specific IgE level but not from eosinophils, indicating that increased FE_{NO} level is mostly reflected in allergic childhood asthma activated by inflammatory cytokines IL-4 and IL-13, but not IL-5.[44] However, caution is warranted when generalizing our results to subjects other than children in this age range, since reports show an age-dependent interaction between sensitization and elevated eosinophil levels in asthma cases.[45] Allergic eosinophilic asthma in children often coexist with allergic sensitization,[46] while the late onset phenotype, non-allergic eosinophilic asthma, is not induced by specific allergens.[46] In addition, the cellular component of inflammation may not be adequately represented by eosinophilic granulocytes since innate lymphoid cells (ILC2) are suggested to be more relevant markers.[47,48]

The major strength of our study is that we used a population-based twin sample of children. We have also used reliable objective biomarkers and the asthma status was based on definitions from the ISAAC study.[26] Another strength of our study is that we included both a continuous and dichotomous IgE variable on sensitization to aeroallergens. This continuous measure enabled us to utilize all the information about the allergen-specific IgE level variable by maximizing the statistical power. Levels below 0.35kU_A/L can provide additional prognostic information, since results have shown that children who show low sensitization (ie 0.10-0.34 kU_A/L) to food allergens in infancy seem to have an increased probability of sensitization to aeroallergens in later life.[29] Furthermore, we adjusted for age, sex and ICS use and we re-weighted the analyses by sampling probability. We assumed

TABLE 3 Results from multivariate modelling

	AE	AE – relative contributions
Total r_{ph}[a]	**0.19 (0.05-0.33)**	NA
$r_{ph\text{-}a}$[a]	0.14 (−0.02 to 0.29)	**0.73 (0.18-1.27)**
$r_{ph\text{-}e}$[a]	0.05 (−0.05 to 0.16)	0.27 (−0.27 to 0.82)
$r_{ph\text{-}a}$: IgE level[b]	**0.10 (0.03-0.18)**	**0.54 (0.03-1.06)**
$r_{ph\text{-}a}$: Eosinophils[b]	0.01 (−0.02 to 0.04)	0.05 (−0.11 to 0.21)
$r_{ph\text{-}a}$: Shared between IgE level and eosinophils[b]	0.02 (−0.01 to 0.06)	0.13 (−0.05 to 0.32)
$r_{ph\text{-}a}$: Asthma and FE_{NO}[a]	−0.00 (−0.15 to 0.15)	−0.00 (−0.78 to 0.78)
$r_{ph\text{-}e}$: IgE level[b]	0.01 (−0.02 to 0.04)	0.07 (−0.11 to 0.24)
$r_{ph\text{-}e}$: Eosinophils[b]	−0.01 (−0.04 to 0.03)	−0.04 (−0.23 to 0.16)
$r_{ph\text{-}e}$: Shared between IgE level and eosinophils[b]	0.00 (−0.01 to 0.02)	0.01 (−0.06 to 0.09)
$r_{ph\text{-}e}$: Asthma and FE_{NO}[a]	0.04 (−0.05 to 0.14)	0.23 (−0.26 to 0.73)

Note: Phenotypic correlation from best-fitting model; due to asthma and FE_{NO} and/or from eosinophils and IgE level. r_{ph} = phenotypic correlation, $r_{ph\text{-}a}$ = part of phenotypic correlation, which is attributable to additive genetic effect, $r_{ph\text{-}e}$ = part of phenotypic correlation, which is attributable to unique environmental effect, IgE level = continuous value of allergen-specific IgE level, NA = not applicable. bold = statistically significantly different from 0.

[a]Biserial correlation.

[b]Pearson correlation.

that the tobacco use would be minor since of the age of the children; thus, we did not include it as a covariate. Limitations include the inherently low power of the classical twin method to detect effects of a shared environment. This may partly explain the absence of shared environmental factors, C, even though we used a population-based twin sample. Factors that are shared within-twin pairs, such as socioeconomic status, that we not did control for, would end up as C in the models. One might further question the generalizability of the results from twin studies to the general population. Twins differ from singletons in that they are, on average, born smaller; however, we have previously shown that, after taking gestational age into account, twins are not at a higher risk of asthma.[49] Studies in epigenetic markers of DNA methylation and gene expression has demonstrated that twins, already at birth, exhibit a large range of epigenetic discordance.[50,51] Thus, twin similarities can also be produced by epigenetic components in addition to the genome.

Asthma is a complex disease characterized by a set of genetically heterogeneous phenotypes. As new phenotypes for asthma are discovered, twin studies provide a first effort in determining the contribution of genetic and environmental factors to these traits.[52] We are not aware of any other studies that have estimated the proportion of covariance by genetic and environmental effects of inflammatory markers (ie allergen-specific IgE level and eosinophils) on the asthma vs. FE_{NO} association. The source of the high variability in individual FE_{NO} levels in asthmatics is largely unknown, but the present study results may give a partial explanation for this heterogeneity. Thus, the biological background of inflammation should be considered for future personalized medicine.

5 | CONCLUSIONS

This study provides further understanding of genetic influence from allergen-specific IgE level, but not blood eosinophils, on the FE_{NO} asthma association. The results presented here shed new light on the he clinical heterogeneity of FE_{NO} values in asthmatic children. As a next step this could encourage omic-studies taking the allergen-specific IgE level into account when investigating inflammation markers in children with asthma.

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CONFLICT OF INTEREST

The authors report no conflict of interest related to the manuscript content.

AUTHOR CONTRIBUTIONS

Anna Hedman: Conceptualization (lead); Formal analysis (equal); Funding acquisition (supporting); Investigation (lead); Methodology (lead); Project administration (lead); Writing-original draft (lead); Writing-review & editing (lead). **Ralf Kuja-Halkola:** Formal analysis (equal); Methodology (equal); Writing-review & editing (equal). **Anne K. Örtqvist:** Data curation (supporting); Investigation (supporting); Project administration (supporting); Validation (supporting); Writing-original draft (equal); Writing-review & editing (equal). **Marianne van Hage:** Conceptualization (supporting); Funding acquisition (supporting); Methodology (equal); Resources (equal); Validation (equal); Writing-original draft (equal); Writing-review & editing (equal). **Catarina Almqvist:** Conceptualization (equal); Funding acquisition (lead); Investigation (equal); Project administration (equal); Resources (equal); Writing-original draft (equal); Writing-review & editing (equal). **Björn Nordlund:** Conceptualization (lead); Formal analysis (supporting); Funding acquisition (equal); Investigation (equal); Project administration (equal); Resources (equal); Writing-original draft (equal); Writing-review & editing (equal).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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