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The STOPPA Twin Study Explains the Exhaled Nitric Oxide and Asthma Link by Genetics and Sensitization

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Background: The link between asthma and exhaled nitric oxide (FENO) is not completely understood. The aim of this study was to estimate the association between FENO and asthma, taking genetics, sensitization, and inhaled corticosteroids (ICS) into account. *Methods:* A total of 681 twins (53% monozygotic [MZ] and 47% dizygotic [DZ]) from the population-based STOPPA study (mean age 12.6 years) were recruited and information on FENO (parts per billion), parental report of current asthma, sensitization to airborne allergens (Phadiatop; IgE $\geq$0.35 kU_A/l), and ICS-treatment was collected. We estimated the association between FENO and asthma, sensitization, and ICS in all twins and within pairs (DZ and MZ) to address shared genetic and environmental factors. Linear regression of log-transformed FENO was used and results presented as exponentiated regression coefficients (exp[β]), with 95% confidence interval (CI). *Results:* We found an association between asthma and FENO in all twins, exp(β) 1.31 [1.11, 1.54]. In within-pairs analysis, the association was stronger within DZ pairs discordant for FENO, exp(β) 1.50 [1.19, 1.89], compared to MZ pairs, exp(β) 1.07 [0.84, 1.37], $p = .049$. There was no difference in FENO in non-sensitized children with asthma, compared to children with neither asthma nor sensitization, exp(β) 0.89 [0.77, 1.03]. However, increased FENO was associated with sensitization, exp(β) 1.48 [1.30, 1.69], and with sensitization together with asthma, exp(β) 1.98 [1.57, 2.51], in all twins and within DZ pairs discordant for FENO, but not in MZ pairs. The FENO asthma association remained in DZ pairs without regular ICS-treatment. *Conclusions:* The association between FENO and asthma is explained by genetics and sensitization.

■ **Keywords:** asthma, exhaled nitric oxide, genetics, sensitization, twins

Asthma is a chronic inflammatory and obstructive airway disease (Simonsson, 1980). In allergic asthma, the inflammation is initiated by immunoglobulin E (IgE) and driven by activation of mast cells and eosinophils (Balzar et al., 2007). Nitric oxide (NO) is an endothelium-derived gas molecule found in exhaled breath (Gustafsson et al., 1991) and a biomarker of airway inflammation (Gratziou et al.,1999). NO is derived from L-arginine by the enzyme NO synthase, which is induced by inflammatory stimuli and inducible NO synthase (Ricciardolo et al., 2004). The fraction of exhaled nitric oxide (FENO) is higher in steroid-naïve subjects with IgE sensitization compared to non-sensitized subjects (Alving et al., 1993), and levels are reduced after intake of inhaled corticosteroids (ICS; van Rensen et al., 1999). The prospective benefit of diagnosing and treating asthma according to FENO is doubtful since coexistence of allergen-specific IgE antibody levels and individual variations seem to confound measurements (Dweik et al., 2010; Strunk et al., 2003). A population-based twin study among adults demonstrated that 60% of

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the variation in FENO between individuals appeared to be explained by genetic factors (Lund et al., 2007). Genetic studies in humans have identified candidate genes responsible for NO synthase regulation determining FENO levels in exhaled breath, both among healthy individuals (Lane et al., 2004) and those with asthma (Salam et al., 2011).

Genetic studies using twin design provide an alternative and powerful approach to improve understanding of asthma origins and expressions (van Dongen et al., 2012). Twin siblings share genes, half if dizygotic (DZ) and all if monozygotic (MZ), some intrauterine exposures, maternal factors, and early environment. This difference in genetic similarity, along with the assumption that twins within a pair raised together share environmental factors — for example, allergen exposure to the same extent irrespective of zygosity — is the basis of twin design studies (Lichtenstein et al., 2002). Twin pairs discordant in FENO are especially powerful to study genetic and environmental influences on FENO manifestations (Morley & Dwyer, 2005; Ortqvist et al., 2009). If associations seen in a cohort of twins remain in within-pair analyses, then factors specific to each individual must be involved in the underlying causal pathways. Conversely, if the relationships disappear or substantially diminish in within-pair analyses, then factors common to the pair must be involved. Genetic influences on the association between FENO and asthma are indicated if associations are stronger within asthma-discordant DZ pairs compared with asthma-discordant MZ pairs, while similar findings in MZ and DZ point to causal effects or environmental explanations. The aim of this study was to analyze the association between FENO and asthma, taking genetics, sensitization, and ICS into account.

Materials and Methods

Study Design and Population

The Swedish Twin Study on Prediction and Prevention of Asthma (STOPPA) is a cross-sectional, population-based twin study on childhood asthma concordant, discordant, and healthy concordant twins (Almqvist et al., 2015). The participants were recruited from the Child and Adolescent Twin Study in Sweden, which contains information on zygosity (80% with DNA markers) and asthma from parental interviews for 9- to 12-year-old twins (Magnusson et al., 2013). Validated questions on asthma (yes/no) and wheezing (current or after 3 years of age yes/no) from the International Study of Asthma and Allergies in Childhood questionnaire (ISAAC) were used to identify twins discordant and concordant for asthma or wheeze (Asher et al., 1995; Supplementary Figure S1). Three groups of MZ and DZ same-sex twin pairs who were raised together were recruited to STOPPA: (1) both twins with asthma or wheezing (asthma concordant), (2) one twin with asthma or wheezing and the other without (asthma discordant), and (3) none of the twins with asthma or wheezing (healthy concordant; Almqvist et al., 2015). The study population included 681 twins with complete information on FENO and asthma, 656 of whom were from complete twin pairs, 53% MZ and 47% same-sex DZ-pairs. Children and parents participating in STOPPA gave informed written consent. The study was approved by Regional Ethical Review Board in Stockholm, Sweden.

Data Collection

At the clinical examination, parents answered a questionnaire on each twin's medical history, including validated questions on asthma disease and treatment (Almqvist et al., 2011; Asher et al., 1995). Parents confirmed asthma and ICS treatment by answering yes to the questions 'Does your child have asthma?' and 'Has your child used ICS treatment regularly during the last 12 months?'

Exhaled nitric oxide was measured during exhalation of at least 6 seconds at flow 50 ml/s (FENO) with a hand-held electrochemical analyzer (NIOX Mino, Aerocrine, Solna, Sweden) according to the guidelines (American Thoracic & European Respiratory, 2005). The average integer value of FENO (parts per billion) was recorded based on two consecutive measures if less than 5% difference or based on three measures if >5% difference.

Serum was collected and analyzed for IgE to Phadiatop® (Thermo Fisher Scientific, Uppsala, Sweden), a screening test for common inhalant allergens (birch, timothy, mugwort, cat, dog, horse, house dust mites [*Dermatophagoides pteronyssinus* and *farina*], and mould [*Cladosporium herbarum*]. Sera that tested positive in Phadiatop®, $\geq$0.35 kU_A/l, were subsequently analyzed for IgE antibodies to the single allergens listed above (ImmunoCAP System, Thermo Fisher Scientific, Uppsala, Sweden). Sensitization was defined as an allergen-specific IgE level of $\geq$0.35 kU_A/l to any of the tested inhalant allergens.

Statistical Analyses

First, to analyze the overall association (among all twins) between FENO, as dependent variable, and asthma, ordinary linear and logistic regression models were applied. Inverse probability weighting was used to account for the sampling procedure, using the different sampling probabilities in each of the six strata (asthma concordant, asthma discordant, and healthy concordant in MZ and DZ, respectively) and the robust standard error estimator was used to account for clustering within twin pairs. The distribution of FENO was skewed and therefore log-transformed when used as a continuous outcome variable (log FENO). The exponentiated regression coefficient, exp(β), is an estimate of the ratio of the geometric mean in appropriate category over the reference category (Bland & Altman, 1996). The (exp[β]-1)×100 is interpreted as the percentage difference between groups. FENO was dichotomized by the 75th percentile in the study population, that is, 18.25 ppb, and used as categorical outcome variable. Robust standard errors

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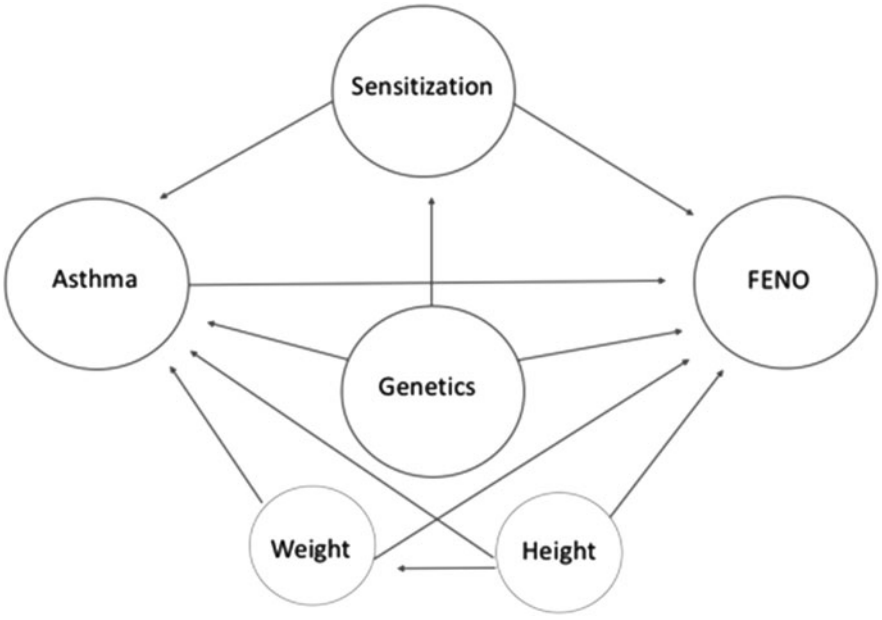

FIGURE 1.
Hypothesized influences for the association between FENO values and asthma in school children.

were used to account for the correlations due to the clustering of observations within twin pairs. Results of regression models were presented as exponantiated regression coefficients (exp[β]) for linear models or odds ratios for logistic models with 95% CI. Second, to estimate the association between FENO as dependent variable and asthma, with adjustment for shared environmental and genetic factors within the twin pairs, we used FENO discordant pairs, that is, pairs with different FENO values within the pair in a fixed effect (conditional) linear and conditional logistic regression model. Twin pairs were FENO discordant if their difference in continuous FENO was equal to or more than 1 ppb, or for the dichotomized FENO variable if one twin was above 18.25 ppb and one below. Third, in a final step, we included interaction terms between asthma and zygosity in the fixed effects regression models in order to test for differential effects for MZ and DZ twins and to estimate zygosity specific effects. If the effect is weaker within MZ twins, where we adjust for 100% of their genes, compared to DZ twins, where adjustment for genes is incomplete (50%), it is evidence of genetic effects underlying the less adjusted association. We estimated the association between FENO and phenotype combinations of asthma and sensitization (neither asthma nor sensitization; asthma but no sensitization; sensitization but no asthma; both asthma and sensitization) and between FENO and asthma with and without regular ICS treatment. Height and weight were identified as potential confounders using directed acyclic graph (Figure 1; Malmberg et al., 2006); however, weight did not change the coefficient (<5%) and was subsequently not included in the final models. Statistical analyses were performed using STATA statistical software (release 13.1; Stata Corp, College Station, TX, USA).

Results

Characteristics of the Study Population

The average age of the study population ($n = 681$) was 12.6 years (*SD* 1.5), and comprised 62% twins with a family history of atopic disease and 53% MZ twins. A total of 54% were males. Characteristics of the study population compared to children with missing information on FENO and current asthma ($n = 71$) are shown in Supplementary Table S1.

Characteristics of Children With Asthma

Children with parental-reported current asthma (19.2%) were on average 3.6 months younger than those without asthma (Table 1). The prevalence of family history of allergic disease and sensitization was more pronounced in children with current asthma than in those without asthma. Mean FENO was higher in children with current asthma compared to children without asthma (24.7 ppb vs. 15.8 ppb, $p < .001$).

Association Between Asthma and FENO

Children with current asthma had increased odds of FENO >18.25 ppb; among all twins adjusted odds ratio (AOR) was 2.55 (95% CI [1.53, 4.24]), and within-twin pairs 5.97 (95% CI, [1.41, 25.2]); see Table 2. In within-pair analysis, the association between FENO and asthma remained in DZ, AOR 5.56 (95% CI [1.16, 26.7]), but not in MZ, AOR 1.49 (95%

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TABLE 1

Background Factors and Clinical Characteristics of Children With and Without Current Asthma in the Study Population, *n* = 681

	Current asthma		No current asthma	
	n	%	*n*	%
Age				
Number (%) of children	131	19	550	81
Mean (*SD*)		12.3 (1.5)		12.6 (1.4)
Sex				
Male	72	55	293	53
Female	59	45	257	47
Family history of atopy*				
No	27	23	190	42
Yes	92	77	262	58
Zygosity				
MZ	75	57	289	53
DZ	56	43	261	47
FENO				
≥ 18.25 ppb.	56	43	115	21
Arithmetic mean (SD)	131	24.7 (21.7)	550	15.8 (13.6)
Geometric mean	131	17.5	550	12.9
Sensitization**				
Negative	45	38	338	66
Positive	74	62	178	35
Wheeze last 12 months				
No	47	41	285	90
Yes	68	59	30	10
Asthma medication				
No	38	29	432	99
Yes	93	71	6	1
ICS*** — regularly				
No	81	66	435	100
Yes	41	34	1	0

Note: *SD* = standard deviation. *Family history of atopic disease: Doctor's diagnose of asthma, eczema or hay fever to pollen or furred animal. **Sensitization: sIgE ≥ 0.35 kU_A/L to any of the tested inhalant allergens (birch, timothy, mugwort, cat, dog, horse, house dust mites (*Dermatophagoides pteronyssinus and farinae*), and mould (*Cladosporium herbarum*)).***Regular use of inhaled corticosteroids (ICS) during the last 12 months.

CI [0.33, 6.74]), although there was no statistical difference between estimates for MZ and DZ twins ($p = .23$).

Linear regression demonstrated a significant association between current asthma and FENO among all twins, with 31% higher FENO in children with asthma, exp(β) 1.31 (95% CI [1.11, 1.54]) compared to those without, and a similar estimate in within-twin pair analysis, exp(β) 1.37 (95% CI [1.14, 1.64]); see Table 3. The association between FENO and asthma within pairs was significantly stronger in DZ compared with MZ ($p = .049$).

Effects of Sensitization and Asthma on FENO

Table 4 shows the association between FENO and asthma and/or sensitization among all twins using linear regression. The FENO in children with asthma without sensitization, exp(β) 0.89 (95% CI [0.77, 1.03]), was comparable to that of children with neither asthma nor sensitization. However, FENO was significantly increased in both children with sensitization only, exp(β) 1.48 (95% CI [1.30, 1.69]), and in combination with asthma, exp(β) 1.98 (95% CI [1.57, 2.51]). This association between sensitization and increased FENO was stronger for children with sensitization and asthma compared to sensitization only ($p = .027$). However, in within-pairs analyses (adjusting for all environmental and genetic factors that are shared within the twin pair), an association between sensitization and increased FENO was noted, but there was no difference between sensitization only and sensitization with asthma ($p = .069$). The associations remained, although attenuated among DZ but not MZ twin pairs, and there was no difference in effect between MZ and DZ twins.

The Association Between Asthma, ICS Treatment, and FENO

Table 5 displays the association between asthma and log FENO in children with ($n = 41$) and without ($n = 81$) regular ICS usage against a reference population without asthma ($n = 550$). Among all twins, FENO levels were significantly increased in children with asthma regardless of ICS. Within all twin pairs, FENO levels remained significantly increased in children with asthma without regular ICS, exp(β) 1.48 (95% CI [1.17, 1.87]), but not in those on regular treatment with ICS, exp(β) 1.10 (95% CI [0.81, 1.49]). This finding was also consistent within DZ pairs, but not in MZ pairs.

Discussion

In this population-based twin study, we found an association between FENO levels and asthma that remained within twin pairs discordant for FENO and was significantly stronger in DZ pairs compared to MZ pairs. This indicates that the association between FENO and asthma is explained by genetic influences and sensitization. The interpretation of this is based on the difference in genetic similarity within DZ pairs compared to MZ pairs, along with the assumption that twins within a pair share environmental factors to the same extent irrespective of zygosity. Our results also demonstrated significantly increased FENO levels in children with sensitization regardless of asthma, but not in those with asthma without sensitization. In particular, FENO values were comparable between children with sensitization only and sensitization with asthma within MZ-twin pairs discordant for asthma and sensitization, which give adjustment for shared environmental factors and all genes. This finding highlights that the inflammatory marker of FENO seems to be more strongly associated with sensitization than with asthma. In addition, we saw that the association between FENO and asthma remained in children without regular treatment with ICS among all twins and within DZ-twins.

Sensitization is present in the majority of school children with asthma and is a predictor of persistent asthma. Moreover, it has been shown to explain approximately 15–60% of a FENO value (Ludviksdottir et al., 2012). The novelty of this twin study is that sensitization, in combination with genetics, explains the link between FENO values and asthma. It is well known that sensitization per se does not confirm

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TABLE 2

Logistic Regression Analysis Showing Association Between Current Asthma and FENO (>18.25 ppb), Among All Twins and Within-Pair Analyses (Conditional Logistic Regression in FENO Discordant Pairs) for All Pairs and Separate Estimates for MZ and DZ With Test of Difference in Within-Pair Estimates by Zygosity (Interaction With Zygosity)

Crude	All twins FENO > 18.25		Within-pair all FENO > 18.25		Within-pair MZ FENO > 18.25		Within-pair DZ FENO > 18.25		Interaction with zygosity
Asthma	*n*	*OR* (95% CI)	*n**	*OR* (95% CI)	*n**	*OR* (95% CI)	*n**	*OR* (95% CI)	*p* value
No	550	1	20	1	7	1	13	1	
Yes	131	2.30 [1.42, 3.72]	20	3.92 [1.30, 11.8]	7	1.50 (0.33, 6.82)	13	5.63 [1.18, 26.9]	.23
Adjusted asthma									
No	550	1	20	1	7	1	13	1	
Yes	131	2.55 [1.53, 4.24]	20	5.97 [1.41, 25.2]	7	1.49 (0.33, 6.74)	13	5.56 [1.16, 26.7]	.23

Note: *Frequencies presented are the number of individuals contributing directly to the estimates of interest, i.e., the number of individuals from pairs that are discordant on both asthma and FENO. **Adjusted for height.

TABLE 3

Linear Regression Analyzing Association Between Current Asthma and FENO, Among All Twins and Within-Pair Analyses (Linear Fixed Effect Regression) for All FENO Discordant Pairs and Separate Estimates for MZ and DZ, With Test of Difference In Within-Pair Estimates by Zygosity (Interaction With Zygosity)

Crude	All twins log FENO		Within-pair all log FENO		Within-pair MZ log FENO		Within-pair DZ pairs log FENO		Interaction with zygosity
asthma	*n*	exp(β) (95% CI)	*n**	exp(β) (95% CI)	*n**	exp(β) (95% CI)	*n**	exp(β) (95% CI)	*p* value
No	550	1	61	1	27	1	34	1	
Yes	131	1.28 [1.09, 1.50]	61	1.37 [1.14, 1.65]	27	1.07 [0.84, 0.37]	34	1.50 [1.19-1.89]	.048
Adjusted** asthma									
No	550	1	61	1	27	1	34	1	
Yes	131	1.31 [1.11, 1.54]	61	1.37 [1.14, 1.64]	27	1.07 [0.84, 1.37]	34	1.50 [1.19-1.89]	.049

Note: Presented values are exponentiated regression coefficients, exp(β) with 95% confidence interval (95% CI), which is an estimate of the ratio of the geometric mean in twins with asthma compared to twins without asthma. *Frequencies presented are the number of individuals contributing directly to the estimates of interest, that is, the number of individuals from pairs that are discordant on both asthma and FENO. **Adjusted for height.

TABLE 4

Linear Regression Examining Association Between FENO and Current Asthma and/or Sensitization Among All Twins and Within-Pair Analyses (Linear Fixed Effects Regression) for All FENO Discordant Pairs and Separate Estimates for MZ and DZ, with Test of Difference in Within Pair Estimates by Zygosity (Interaction With Zygosity)

Crude		All twins log FENO		Within-pair all log FENO		Within-pair MZ log FENO		Within-pair DZ log FENO		Interaction with zygosity
asthma	Sensitization	*n*	exp(β) (95% CI)	*n**	exp(β) (95% CI)	*n**	exp(β) (95% CI)	*n**	exp(β) (95% CI)	*p* value
No	No	338	1	85	1	35	1	50	1	1
Yes	No	45	0.87 [0.75, 1.00]	25	1.00 [0.78, 1.27]	13	1.05 [0.83, 1.33]	12	0.96 [0.66, 1.40]	.69
No	Yes	178	1.48 [1.29, 1.69]	76	1.39 [1.14, 1.70]	34	1.49 [1.09, 2.03]	42	1.33 [1.02, 1.72]	.58
Yes	Yes	74	1.94 [1.55, 2.42]	36	1.86 [1.38, 2.51]	14	1.50 [0.96, 2.35]	22	1.96 [1.36, 2.82]	.36
Adjusted** asthma	Sensitization									
No	No	338	1	85	1	35	1	50	1	1
Yes	No	45	0.89 [0.77, 1.03]	25	0.99 [0.78, 1.27]	13	1.05 [0.83, 1.34]	12	0.96 [0.66, 1.40]	.69
No	Yes	178	1.48 [1.30, 1.69]	76	1.39 [1.15, 1.69]	34	1.49 [1.09, 2.03]	42	1.33 [1.03, 1.72]	.58
Yes	Yes	74	1.98 [1.57, 2.51]	36	1.86 [1.38, 2.51]	14	1.50 [0.96, 2.35]	22	1.96 [1.36, 2.82]	.36

Note: Presented values are expressed as exponentiated regression coefficient, exp(β), with 95% confidence interval (95% CI), which is an estimate of the ratio of the geometric mean in each asthma/sensitization category to the category with neither asthma nor sensitization. *Frequencies presented are the number of individuals contributing directly to the estimates of interest, that is, the number of individuals from pairs that are discordant on both asthma/sensitization and FENO. **Adjusted for height. Sensitization: sIgE $\geq$ 0.35 kU_A/L to any of the tested inhalant allergens (birch, timothy, mugwort, cat, dog, horse, house dust mites (*Dermatophagoides pteronyssinus and farinae*), and mould (*Cladosporium herbarum*)).

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TABLE 5

Linear Regression Showing the Association Between FENO and Current Asthma With and Without Regular Treatment of ICS, Among All Twins and Within-Pair Analyses (Linear Fixed Effect Regression) in all FENO Discordant Pairs and Separate Estimates for MZ and DZ, With Test of Difference in Within-Pair Estimates by Zygosity (Interaction With Zygosity)

Crude		All twins log FENO		Within-pair all log FENO		Within-pair MZ log FENO		Within-pair DZ log FENO		Interaction with zygosity
asthma	ICS	*n*	exp(β) (95% CI)	*n**	exp(β) (95% CI)	*n**	exp(β) (95% CI)	*n**	exp(β) (95% CI)	*p* value
No	No	550	1	57	1	24	1	33	1	1
Yes	No	81	1.31 [1.07, 1.62]	43	1.48 [1.17, 1.87]	16	1.01 [0.81, 1.26]	27	1.66 [1.23, 2.23]	.009
Yes	Yes	41	1.29 [1.00, 1.65]	22	1.10 [0.81, 1.49]	8	1.05 [0.59, 1.85]	14	1.15 [0.81, 1.64]	.78
Adjusted** asthma	ICS									
No	No	550	1	57	1	24	1	33	1	1
Yes	No	81	1.32 [1.07, 1.63]	43	1.48 [1.17, 1.87]	16	1.01 [0.80, 1.26]	27	1.66 [1.23, 2.23]	.009
Yes	Yes	41	1.38 [1.08, 1.76]	22	1.10 [0.81, 1.49]	8	1.04 [0.59, 1.85]	14	1.15 [0.80, 1.63]	.79

Note: Presented values are expressed as exponentiated regression coefficient, exp(β), with 95% confidence interval (95% CI), which is an estimate of the ratio of the geometric mean in each asthma/ICS category to the category with neither asthma nor ICS. *Frequencies presented are the number of individuals contributing directly to the estimates of interest, that is, the number of individuals from pairs that are discordant on both asthma/ICS and FENO. **Adjusted for height.

allergic disease or bronchial inflammation (Blomme et al., 2013), that there is evidence supporting different genetic determinants of sensitization and asthma (Palmer et al., 2000), and that bronchial hyper-responsiveness (BHR) is genetically distinct from atopy (Thomsen et al., 2009). The clinical implications of our study are that sensitization should be examined in individuals with increased FENO values. Moreover, instead of diagnosing asthma based on FENO values, this study indicates that the measurement should rather be used for monitoring patients with asthma.

Studies suggest that FENO is a useful biomarker to measure and monitor asthma in individual subjects in relation to the variability of the disease, asthma control (Stern et al., 2011), and especially during asthma exacerbations (van der Valk et al., 2012). In a twin study, it was proposed that approximately 60–67% of the variation in measured NO in adults could be explained by genetic factors (Lund et al., 2007; Thomsen et al., 2009). However, Lund et al. (2007) found that genetic factors mainly explained the association between NO and BHR (Lund et al., 2007). Although their population-based sample was not powered for further stratification based on atopy discordance and concordance, the association between NO and BHR was stronger among those with atopy compared with those without atopy, which is in line with our findings.

One important factor affecting FENO values is anti-inflammatory treatment with ICS, which decreases FENO rapidly, and subsequently increases FENO after withdrawal (van Rensen et al., 1999). In our study, the effect of genetic influences on the association between FENO and asthma was linked to children without regular ICS treatment. The FENO-asthma association was estimated for within-pairs discordant on both asthma/ICS and FENO, and it was significantly stronger in DZ pairs than in MZ pairs. The individual response to ICS may vary according to environmental factors and disease severity. Perez-de-Llano et al. (2010) suggested that FENO is useful to identify individuals with difficult-to-treat asthma with the potential to respond to high doses of inhaled corticosteroids or systemic steroids. However, the responders of anti-inflammatory treatment in the Perez-de-Llano study were mainly sensitized, and that could explain the effect on FENO values.

The strengths of this study were the twin-design with within-pair analyses, which by design adjust for determinants of FENO that are shared within twin pairs, both unknown and unmeasurable, and known determinants like sex, age, and genetics. Furthermore, the children were examined objectively: FENO was measured according to ATS/ERS guidelines (American Thoracic & European Respiratory, 2005), and analyzed as both categorical and linear measures; information on current asthma was based on definitions from the ISAAC study (Asher et al., 1995); and reliable measures of zygosity were based on a validated algorithm or confirmed by DNA testing (Lichtenstein et al., 2002). A potential limitation of this study was the cross-sectional design and sample size. The study was not large enough to perform reliable within-pair analyses of asthma and sensitization phenotypes stratified by zygosity using the dichotomized FENO measure. However, comparisons could be done using FENO as a continuous outcome measure. Another limitation was the generalizability of results from twin studies to the singleton population. While twins differ from singletons in that they on average are born smaller, we have recently shown that after taking gestational age into account, twins are not at higher risk of asthma (Ullemar et al., 2015).

This twin-designed study concludes that the FENO asthma association is explained by genetics and sensitization, and is influenced by ICS treatment. The clinical implication is to properly assess sensitization and adherence to ICS in children with elevated FENO values.

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Authors' Contributions

All authors — Björn Nordlund, Cecilia Lundholm, Vilhelmina Ullemar, Marianne van Hage, Anne K. Örtqvist, and Catarina Almqvist — have contributed to the conception of the work, and critically revised it for important intellectual content, and approved the final version to be published, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Conflicts of Interest

None.

Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national international committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Supplementary material

To view supplementary material for this article, please visit https://doi.org/10.1017/thg.2017.35

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