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Exhaled nitric oxide, susceptibility and new-onset asthma in the Children's Health Study

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ABSTRACT: A substantial body of evidence suggests an aetiological role of inflammation, and oxidative and nitrosative stress in asthma pathogenesis. Exhaled nitric oxide fraction (*F*eNO) may provide a noninvasive marker of oxidative and nitrosative stress, and aspects of airway inflammation. We examined whether children with elevated *F*eNO are at increased risk for new-onset asthma.

We prospectively followed 2,206 asthma-free children (age 7–10 yrs) who participated in the Children's Health Study. We measured *F*eNO and followed these children for 3 yrs to ascertain incident asthma cases. Cox proportional hazard models were fitted to examine the association between *F*eNO and new-onset asthma.

We found that *F*eNO was associated with increased risk of new-onset asthma. Children in the highest *F*eNO quartile had more than a two-fold increased risk of new-onset asthma compared to those with the lowest quartile (hazard ratio 2.1, 95% CI 1.3–3.5). This effect did not vary with the child's history of respiratory allergic symptoms. However, the effect of elevated *F*eNO on new-onset asthma was most apparent among those without a parental history of asthma.

Our results indicate that children with elevated *F*eNO are at increased risk for new-onset asthma, especially if they have no parental history of asthma.

KEYWORDS: Airway inflammation, exhaled nitric oxide, incident asthma

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Asthma is the most common childhood chronic disease and studies have documented its rise in prevalence over the past several decades [1]. Although the aetiology of asthma has been extensively studied, the pathogenesis and the factors causing the rapid rise in prevalence have yet to be firmly established. To reduce the burden from asthma, more research that focuses on asthma pathogenesis is needed. The current understanding of the pathogenesis of asthma suggests that oxidative and nitrosative stress, and dysregulated inflammatory responses play a role in asthma aetiology [2, 3]. Exhaled nitric oxide fraction (*F*eNO) provides a noninvasive marker of oxidative and nitrosative stress, and aspects of airway inflammation that may have a role in childhood asthma and allergic airway disease pathogenesis [4, 5].

The potential usefulness of *F*eNO in studies of asthma aetiology is illustrated by a recent Swedish population-based study of healthy adults without respiratory symptoms that found that elevated *F*eNO predicted the development of wheeze [6].

The Children's Health Study (CHS), a longitudinal population-based study of respiratory health among school-age children in 13 communities in Southern California, USA, provided an opportunity to investigate whether children with elevated *F*eNO are at increased risk of new-onset asthma. We also hypothesised that the elevated risk of new-onset asthma associated with *F*eNO differs with the child's allergic status and parental history of asthma. We examined the association of new-onset asthma with *F*eNO using data collected annually in 2004–2007 from a cohort of 2,206 children whose parents did not report a physician diagnosis of asthma at study entry.

METHODS

Study subjects

Participants were from a CHS cohort enrolled during 2002–2003 when they were in kindergarten or first grade (average 5–6 yrs old). Informed consent from a parent or guardian and assent from each child were obtained before *F*eNO testing. The University of Southern California's Institutional Review Board (Los Angeles, CA, USA) approved

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the protocol. Parents completed an annual self-administered questionnaire that included sociodemographic and child health characteristics, and a brief exposure history, including exposure to second-hand tobacco smoke and *in utero* exposure to maternal smoking. During 2004–2005, parental consent was obtained and F_{eNO} testing was performed on 2,585 (82%) subjects out of 3,146 eligible cohort members. We further excluded children whose parents reported a physician diagnosis of asthma during the school year of F_{eNO} testing (n=62), children who were lost to follow-up during the year after F_{eNO} testing (n=241) and children whose breath samples were invalidated due to storage or technical problems with the analyser (n=76), resulting in 2,206 subjects.

New-onset asthma definition

An incident asthma case was defined as a child with no prior parental report of a physician diagnosis of asthma at F_{eNO} testing whose parent reported a physician diagnosis of asthma in an annual follow-up questionnaire during the 3-yr follow-up period.

Sociodemographic and medical history information

Race/ethnicity was defined as non-Hispanic white, Hispanic, African-American, Asian/Hawaiian/Pacific Islander or mixed/other ethnicities, based on parental report. Education was defined as the highest level of education attainment of the parent or guardian who completed the questionnaire. Annual household income was used to assess the role of socioeconomic status. We dichotomised self-reported health insurance coverage to assess the role of access to health care.

At study entry, selected aspects of the child's and parents' medical histories were collected, and in each successive school year, an update questionnaire inquiring about the child's intervening year of health was completed by parents and returned to study staff. Child's history of respiratory allergy included any hay fever or allergic rhinitis. Child's history of ever wheezing and wheezing in the past 12 months were defined as ''yes'' or ''no''. Parental history of asthma was defined as an asthma diagnosis in either biological parent. During annual school visits, subjects' height and weight were measured using standardised protocols.

F_{eNO} collection and analysis

Details of breath collection and F_{eNO} analysis used in this study were reported previously [7, 8]. In brief, offline breath collection was performed in the morning at schools to avoid traffic-related peaks of ambient nitric oxide and possible effects of recent eating, according to American Thoracic Society recommendations [9, 10]. Each CHS community was visited at least twice in different seasons, in order to minimise confounding of location and season effects. Health status at testing was evaluated by questionnaire; subjects with symptoms of acute respiratory infection within the past 3 days were excluded or rescheduled. Exhaled breath samples for offline testing were obtained using Bag Collection and Sampling Kits and 1.5-L aluminised Mylar bags (Sievers Division, GE Analytical Instruments, Boulder, CO, USA) by the deadspace-discard method at 100 $mL \cdot s^{-1}$ expiratory flow, stored in temperature-controlled coolers, and transported to and analysed at a central laboratory (Sievers Model 280i chemiluminescent NO analyser). Indoor air samples were also collected to estimate subjects' ambient nitric oxide exposure at testing. Lag times between collection and analysis ranged from 2 to 26 h. When this study began, offline collection was the most feasible method of collection for large field studies. Excellent agreement has been demonstrated between offline and online measurements in laboratory-based studies, using a variety of techniques [11–13]. In this study, offline F_{eNO} values were converted to online F_{eNO} values for all children, as would be measured at 50 $mL \cdot s^{-1}$ expiratory flow [10], using a prediction model (model adjusted $r^2=0.94$) determined in a later substudy of 362 children with concurrent online and offline F_{eNO} measurements [7]. The substudy included one or two testing days at each of 15 schools in eight communities in order to cover most of the geographic and seasonal range. Online measurements were performed at 50 $mL \cdot s^{-1}$ expiratory flow using EcoMedics CLD-88-SP analysers, with DeNOx accessories to provide nitric oxide-free inhaled air (EcoPhysics Inc., Ann Arbor, MI, USA), according to the manufacturer's instructions based on professional societies' recommendations [4, 9–10]. The prediction model included adjustment for ambient nitric oxide concentration, and lag time between collection and analysis. We used predicted online F_{eNO} (hereafter, F_{eNO}) in subsequent analyses.

Statistical methods

In order to investigate the relationship of F_{eNO} with new-onset asthma, we calculated incidence rates and conducted descriptive analyses, and explored a series of multivariate modelling approaches in order to account for potential confounders and heterogeneity of effects within subgroups of children. Crude incidence rates for new-onset asthma were calculated by dividing the number of cases by the total person-years at risk. For children who developed new-onset asthma, follow-up was considered complete at the time of reported diagnosis. Incidence rates were calculated for age-specific quartiles of F_{eNO}. We have previously shown that F_{eNO} varies with age [8]. Therefore, age-specific F_{eNO} quartiles were computed for each of three age strata (defined as <8, 8–9 and >9 yrs) for analyses (see table S1 in the online supplementary material).

To further investigate the association between F_{eNO} and new-onset asthma, we fitted Cox proportional hazards models with sex- and age-specific baseline hazards (with age defined as integer age at F_{eNO} testing). All models were adjusted for community of residence and race/ethnicity to account for the study design, and we assessed potential confounders identified *a priori*, including parental education, annual family income, health insurance, parental history of asthma, body mass index (BMI), household pets or pests, humidifier use, average outdoor air pollution levels on the day of the F_{eNO} measurement (nitrogen dioxide, ozone, and particles with a 50% cut-off aerodynamic diameter of 2.5 and 10 μm), lifetime second-hand smoke (SHS) exposure and *in utero* exposure to maternal smoking. Covariates were considered confounders if the hazard ratio changed by 10% after addition to the base model. The final model was additionally adjusted for lifetime history of wheezing. Heterogeneity of associations among subgroups was assessed by fitting models with appropriate interaction terms, and statistical significance was tested by partial likelihood ratio tests [14]. Stratified analyses were

performed in the presence of significant interaction (p-value <0.05). The nature of the nonlinearity in F_{ENO} effects was explored using splines, piecewise cubic polynomials that are joined smoothly at a number of breakpoints known as knots [15].

Sensitivity analyses were conducted by limiting the asthma case definition to those 1) reporting a diagnosis >1 yr after F_{ENO} testing, 2) without a history of respiratory allergy and 3) reporting use of inhaled medication in the diagnosis year follow-up questionnaire. To explore the role of wheezing prior to the onset of asthma, we restricted the analysis to children without a history of ever wheezing and wheezing 12 months prior to F_{ENO} testing.

All analyses were conducted using SAS software (SAS Institute, Cary, NC, USA) version 9.1. All hypothesis testing was conducted assuming a 0.05 significance level and a two-sided alternative hypothesis.

RESULTS

Study population and cohort follow-up

Descriptive analyses are presented in table 1. There were approximately equal numbers of males and females, and sex was not associated with new-onset asthma. Nearly 50% of children were 8–9 yrs of age at the initial F_{ENO} measurement. The population was ethnically diverse: 55% of the participants were Hispanic white. This was largely a middle-class population: the majority of children lived in households earning more than US$50,000 per year, had health insurance and had parents with at least some university education. None of these characteristics was associated with new-onset asthma.

We ascertained 129 cases of new-onset asthma over a 3-yr follow-up period (69 females and 60 males). The overall crude incidence rate was 22.2 per 1,000 person-yrs (see table S2 in the online supplementary material for rates by sex, ethnicity and other select characteristics). The overall mean and median follow-up times were 2.59 and 2.93 yrs, respectively, and ~25% of the participants were lost prior to the end of follow-up. The proportion of possible follow-up time did not vary substantially by sex, ethnicity or F_{ENO} quartile; however, there were small but significant differences in loss to follow-up rates with respect to parental education, family income and child's health insurance coverage (data not shown). Based on telephone interviews conducted in the CHS with the families of subjects who left the study schools, loss to follow-up was primarily due to family moves out of the school catchment area related to a change in employment [16].

Selected health and exposure characteristics, and risk of new-onset asthma

50% of the participants reported having no history of respiratory allergy at study entry; the remaining 50% were split evenly between past history of respiratory allergy (history of hay fever or allergic rhinitis but no current symptoms) and current respiratory allergy (symptoms within the previous 12 months) (table 1). Children with any history of respiratory allergy showed an increased risk of new-onset asthma relative to the never-allergy group. Any history of wheezing and wheezing in the 12 months prior to F_{ENO} testing were present in 27% and 5% in the participants, respectively, and were associated with a nearly five-fold increased risk of new-onset asthma. 16% reported a parental history of asthma which was associated with a two-fold increased risk of new-onset asthma in the child.

TABLE 1 Subject characteristics and associations with new-onset asthma

Subject characteristics	Subjects[#] n (%)	HR[¶] (95% CI)
Total subjects	2206 (100)	
Females	1155 (52)	1.01 (0.71–1.44)
Age at F_{ENO} testing		
<8 yrs	699 (32)	0.77 (0.48–1.25)
8–9 yrs	1064 (48)	0.74 (0.48–1.16)
>9 yrs	443 (20)	1
Race/ethnicity		
Non-Hispanic White	788 (36)	1
Hispanic	1212 (55)	0.92 (0.60–1.41)
African-American	36 (2)	1.82 (0.62–5.31)
Asian/Hawaiian/Pacific Islander	67 (30	0.64 (0.20–2.09)
Other	101 (5)	1.30 (0.61–2.78)
Allergic status at study entry		
Never	1104 (50)	1
Former hayfever or allergic rhinitis	543 (250	1.65 (1.05–2.60)
Current hayfever or allergic rhinitis	557 (25)	2.38 (1.57–3.60)
History of wheeze		
Never	1598 (73)	1
Lifetime wheeze	604 (270	4.85 (3.38–6.96)
No wheeze 12 months prior to study entry	1983 (95)	1
Wheeze 12 months prior to study entry	110 (5)	4.95 (3.12–7.86)
Any family history of asthma	317 (16)	1.99 (1.32–3.01)
Exposure to SHS	206 (11)	0.99 (0.55–1.80)
***In utero* exposure to maternal smoking**	131 (6)	0.87 (0.40–1.90)
Annual household income US$		
<15000	268 (14)	1.29 (0.69–2.40)
15000–49999	594 (32)	1.04 (0.64–1.69)
⩾50000	1003 (54)	1
No health insurance	260 (13)	1.14 (0.64–2.03)
Parent education level		
<12th grade	425 (21)	0.92 (0.43–1.99)
12th grade	368 (18)	0.73 (0.35–1.53)
Some university	754 (36)	1.04 (0.58–1.87)
University	284 (14)	0.86 (0.43–1.74)
Some postgraduate	242 (12)	1
BMI percentile category		
Underweight *i.e.* <5th percentile	54 (2)	2.05 (0.82–5.14)
Normal weight *i.e.* 5th to <85th percentile	1371 (62)	1
Overweight/obese *i.e.* ⩾85th percentile	781 (35)	1.44 (0.99–2.08)

HR: hazard ratio; F_{ENO}: exhaled nitric oxide fraction; SHS: second-hand smoke; BMI: body mass index. [#]: numbers may not sum to 2,206 due to missing values; [¶]: adjusted for race/ethnicity and community with baseline strata for age and sex (where appropriate).

62% of study participants had normal BMI at baseline. Neither underweight (<5th BMI percentile) nor overweight/obese (⩾85th percentile) was significantly associated with increased risk of new-onset asthma. Neither lifetime SHS exposure nor *in utero* exposure to maternal smoking was associated with risk of new-onset asthma. Prevalence of SHS exposure and *in utero*

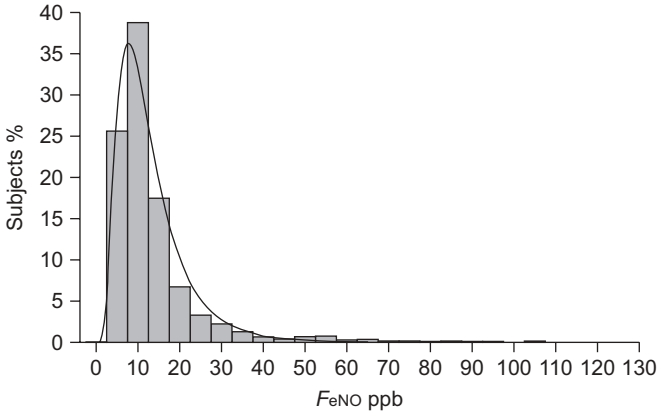

FIGURE 1. Distribution of exhaled nitric oxide fraction (*F*eNO) at baseline (mean±SD 13.6±12.0 ppb; n=2,206).

exposure to maternal smoking in this population was lower than in previous CHS cohorts [17] (11% and 6%, respectively).

Distribution of FeNO

As we previously reported [8], *F*eNO followed an approximately log-normal distribution (mean 13.6 ppb; median 10.1 ppb; SD 12.0 ppb; range 2.3–132.4 ppb) (fig. 1). The median concentrations of *F*eNO (at baseline) among subjects who developed new-onset asthma was 10.9 ppb (range 3.2–132.4 ppb) *versus* 10.1 ppb (range 2.3–107.2 ppb) among subjects who did not develop asthma.

FeNO and risk of new-onset asthma

Elevated *F*eNO was associated with an increased risk of new-onset asthma (table 2 and fig. 2). Children with *F*eNO in the highest quartile at the start of follow-up had more than a two-fold increased risk of incident asthma compared to those with *F*eNO in the lowest quartile (HR 2.11, 95% CI 1.26–3.51), after adjusting for race/ethnicity, community of residence and lifetime history of wheeze. We observed an increasing trend of asthma risk with increasing quartiles of *F*eNO (p-value for trend <0.01). The association of new-onset asthma with *F*eNO was not substantially affected by adjustment for parental education, family income, health insurance, family history of asthma, household pets or pests, humidifier use, BMI, daily average air pollution levels (on the day of *F*eNO measurement) SHS exposure or *in utero* exposure to maternal smoking (data not shown).

To assess the role of past wheezing, we adjusted the risk estimates for wheezing history and conducted analyses among children without any history of wheezing. Adjustment for any history of wheezing changed the risk estimates by slightly more than 10%. In sensitivity analyses restricting the cohort to children without a history of wheezing (n=1,602) or without wheezing in the 12 months prior to *F*eNO measurement (n=2,096), we found a similar increased risk of new-onset asthma for those with the highest quartile of *F*eNO (table 3).

To assess the effect of asthma case definition, we conducted analyses restricting cases to those with recent inhaled medication use. We found that the estimates for the effects of *F*eNO on new-onset asthma were larger (over four-fold risk comparing the highest to the lowest quartile of *F*eNO; table 4 and online supplementary table S3). To investigate the contribution of delayed asthma diagnosis, we restricted the analysis to follow-up starting in the second year and found that eliminating the first year of follow-up did not substantially alter the relative risk estimates for *F*eNO (table 4).

FeNO and new-onset asthma by allergy status and parental history of asthma

In contrast to our hypothesis, we did not find evidence that the effect of *F*eNO on new-onset asthma depended on respiratory allergy status (table 5). The pattern of increasing risk of new-onset asthma with increasing *F*eNO was observed in children with and without a reported history of respiratory allergy.

The effect of *F*eNO on new-onset asthma differed among children with and without a parental history of asthma (table 6 and online supplementary fig. S1; p-value for interaction <0.05). The observed increase in risk of asthma development was most apparent among children without a parental history of asthma. Compared with children with the lowest *F*eNO quartile with no parental history of asthma, children in the highest *F*eNO quartile with no parental history of asthma had more than a three-fold increased risk of new-onset asthma (HR 3.18, 95% CI 1.66–6.08). This pattern of increasing risk with increasing *F*eNO was observed among children with either maternal or paternal asthma (data not shown). Although parental history of asthma was directly associated with elevated risk of new-onset asthma, we observed little evidence for association of increasing *F*eNO with increasing risk of

TABLE 2 Association of exhaled nitric oxide fraction (*F*eNO) with new-onset asthma in the Children's Health Study

Age-specific quartiles of *F*eNO at baseline	New-onset asthma	No asthma	HR# (95% CI)
All subjects n	129	2077	
Quartile 1	24 (19)	528 (25)	1
Quartile 2	30 (23)	521 (25)	1.53 (0.89–2.63)
Quartile 3	30 (23)	523 (25)	1.68 (0.97–2.90)
Quartile 4	45 (35)	505 (24)	2.11 (1.26–3.51)

Data are presented as n (%), unless otherwise stated. HR: hazard ratio. #: adjusted for race/ethnicity, lifetime wheeze and community with baseline strata for age and sex. p-value for trend <0.01.

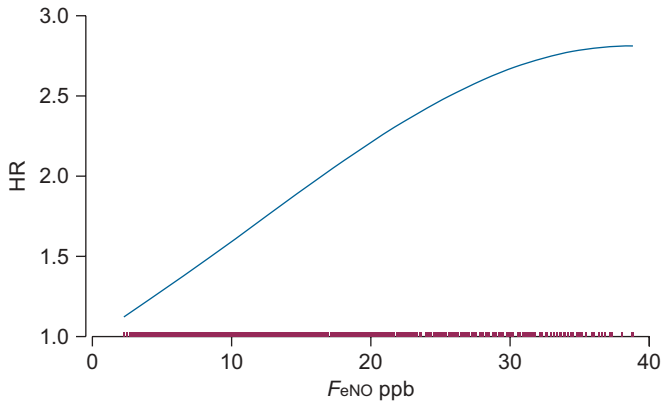

FIGURE 2. Hazard ratio (HR) function of the effect of exhaled nitric oxide fraction (*F*eNO) on new-onset asthma. The model was fit for subjects with *F*eNO ⩽40 ppb (~95% of subjects) due to sparseness of data above 40 ppb.

new-onset asthma in children with a parental history of asthma (although power was limited by sample size and number of cases in this group).

In further analyses, the association of *F*eNO with new-onset asthma did not differ in males or females, children of different ethnicity, those exposed to SHS, or those exposed *in utero* to maternal smoking (data not shown).

DISCUSSION

We found that children with higher *F*eNO had a substantial risk of incident asthma compared with children with low *F*eNO. The use of *F*eNO in asthma clinical practice has been extensively investigated [18]. While the role of *F*eNO in clinical practice remains unclear [19], a number of studies have supported the use of *F*eNO in monitoring adherence to medication [20], maintaining asthma control and predicting relapse [21, 22]. Especially in the presence of symptoms, elevated *F*eNO (*e.g.* >35 ppb in steroid-naïve patients) has been supportive of asthma diagnosis [18, 23]. However, to our knowledge, this is the first investigation to demonstrate the predictive value of *F*eNO for identifying children at risk for developing asthma, thereby extending the utility of this marker beyond monitoring medication adherence, predicting asthma exacerbations or verifying a diagnosis.

A number of studies have identified a subgroup of individuals with elevated *F*eNO without asthma or asthma-related symptoms [24–27]. In a cross-sectional study of 13–14-yr-old schoolchildren, NORDVALL *et al.* [27] suggested that a small subset of participants with elevated levels of *F*eNO who reported no symptoms of asthma in the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire [28] may represent ''early asthma''. SIVAN *et al.* [29] compared the use of *F*eNO, spirometry and induced sputum eosinophil count in consecutive school-age children referred for evaluation of possible asthma. The sensitivity, specificity, and positive and negative predictive values for best cut-off points of *F*eNO (19 ppb) were all >80% and were very similar to those for sputum eosinophil count, suggesting that *F*eNO testing is about as effective as sputum induction in aiding the diagnosis of childhood asthma.

In a population-based prospective study of adults who were free of asthma or wheeze at study entry, OLIN *et al.* [6] found that baseline *F*eNO over the 90th percentile predicted new-onset wheeze at 4-yr follow-up among adults. Because their study was underpowered to investigate new-onset asthma, the authors used new-onset wheeze as a surrogate and early marker of asthma. Regardless of wheeze history, elevated *F*eNO was predictive of new-onset asthma in our population of schoolchildren.

It is widely accepted that genetic factors account for a significant proportion of allergy and asthma occurrence [30, 31]. In this study, we found nearly a two-fold increased risk of new-onset asthma in children with a parental history of asthma; however, the size of that risk did not vary significantly by quartiles of *F*eNO. The effect of elevated *F*eNO on new-onset asthma was more marked among children without a parental history of asthma. We previously reported that 23% of the parent cohort indicated a parental history of asthma at cohort establishment [32] (2 yrs before *F*eNO measurement). The prevalence of parental asthma in the current study was 16%, probably due to the exclusion of prevalent asthma cases and cases diagnosed in the 2 yrs before *F*eNO measurement. Moreover, the proportion of cases with a parental history of

TABLE 3 Exhaled nitric oxide fraction (*F*eNO) and risk of new-onset asthma, restricted to children without lifetime wheezing and without wheezing in the 12 months prior to study entry

Age-specific quartiles of *F*eNO at baseline	No lifetime history of wheezing#	No wheezing in 12 months prior to study entry¶
Subjects n	1602	2096
Quartile 1	1	1
Quartile 2	2.09 (0.89–4.95)	1.70 (0.95–3.06)
Quartile 3	0.99 (0.36–2.66)	1.41 (0.76–2.63)
Quartile 4	2.42 (1.00–5.86)	2.19 (1.22–3.90)
ptrend	0.19	0.02

Data are presented as hazard ratio (95% CI), unless otherwise stated. ptrend: p-value for trend. #: adjusted for race/ethnicity and community, and stratified by integer age and sex in children without a history of wheezing (48 cases and 1,554 noncases); ¶: adjusted for race/ethnicity and community, and stratified by integer age and sex in children without reported wheezing in the 12 months prior to study entry (105 cases and 1,991 noncases).

TABLE 4 Exhaled nitric oxide fraction (F_{ENO}) and risk of new-onset asthma: restricted case definitions

	Restricted to cases reporting a diagnosis after >1 yr of follow-up	Restricted to cases with no history of allergy	Restricted to cases reporting recent inhaled medication use	Restricted to cases categorised as moderate or severe
Subjects n	2154	2120	2133	2142
Cases n	77	43	56	65
Age-specific quartiles of F_{ENO} at baseline				
Quartile 1	1	1	1	1
Quartile 2	1.61 (0.78–3.31)	1.59 (0.63–3.99)	3.01 (1.14–7.89)	1.45 (0.58–3.60
Quartile 3	2.09 (1.29–4.25)	1.68 (0.66–4.27)	3.57 (1.35–9.44)	2.21 (0.94–5.60)
Quartile 4	2.17 (1.09–4.34)	1.90 (0.77–4.72)	4.29 (1.70–10.80)	4.22 (1.97–9.02)
p_{trend}	0.02	0.18	<0.005	<0.0001

Data are presented as hazard ratio (95% CI), unless otherwise stated. Adjusted for race/ethnicity, community and lifetime wheeze, and stratified by integer age and sex. Recent inhaled medication use was defined as any rescue or controller medication in the previous 12 months, reported on the diagnosis year follow-up questionnaire. Moderate-to-severe asthma (65 cases) was defined as at least one attack of wheezing in the past 12 months or waking at night due to wheezing in the past 12 months (in the year of diagnosis on the follow-up questionnaire); 24 of these cases were defined as severe, which were classified as four or more wheeze attacks in the previous 12 months, one or more nights per week awakened with wheeze, or limited speech due to wheezing in the previous 12 months (in the diagnosis year on the follow-up questionnaire); due to the small numbers of severe cases, moderate and severe cases were combined in the analysis. p_{trend}: p-value for trend.

asthma was quite similar by child's history of allergy; 29% of allergic and 24% of nonallergic cases had a parental history of asthma (data not shown).

While our analysis is based on small numbers, the absence of an increased risk in children with higher F_{ENO} and a parental history of asthma (relative to children with lower F_{ENO} and a parental history of asthma) may indicate that the new-onset asthma associated with F_{ENO} is not mediated by the same pathways that account for the asthma in children with a parental history of asthma. Alternatively, our study may demonstrate that, beyond the age of 5–8 yrs, the impact of parental history on the development of asthma may be reduced.

Elevated inflammation and oxidative/nitrosative stress could also arise from exposure and/or susceptibility to environmental stressors, such as SHS or ambient air pollution. We have previously reported that the effects of air pollution on asthma risk may differ in children with and without a parental history of asthma. In a CHS cohort recruited in the 1990s, we showed that children who exercised heavily in high-ozone environments were at increased risk of new-onset asthma especially in the absence of a parental history of asthma [33]. We also demonstrated that traffic-related pollution was associated with a two-fold increased risk of lifetime asthma in children without a parental history of asthma [32]. Taken together, our results and previous findings support an

TABLE 5 Association of exhaled nitric oxide fraction (F_{ENO}) with new-onset asthma by respiratory allergy status

Age-specific quartiles of F_{ENO} at baseline	History of respiratory allergy					
	Never			Current or former		
	New-onset asthma	No asthma	HR[#] (95% CI)	New-onset asthma	No asthma	HR[#] (95% CI)
Quartile 1	8	259	1	16	269	1.20 (0.51–2.84)
Quartile 2	11	310	1.30 (0.52–3.23)	19	211	2.10 (0.91–4.85)
Quartile 3	11	280	1.67 (0.66–4.20)	19	243	2.01 (0.87–4.63)
Quartile 4	13	212	2.21 (0.90–5.38)	32	293	2.44 (1.10–5.39)
p_{trend}			<0.05[¶]			<0.05[¶]
$p_{interaction}$			0.89[+]			

Data are presented as n, unless otherwise stated. HR: hazard ratio; p_{trend}: p-value for trend; $p_{interaction}$: p-value for interaction. [#]: adjusted for race/ethnicity, lifetime wheeze and community with baseline strata for age and sex; [¶]: trend tests conducted in stratified models; [+]: based on the Chi-squared statistic using the likelihood ratio test to compare a model with base terms to a model also containing the multiplicative interaction term.

TABLE 6 Association of exhaled nitric oxide (*F*eNO) with new-onset asthma by parental history of asthma

Age-specific quartiles of *F*eNO at baseline	Parental history of asthma					
	No			Yes		
	New-onset asthma	No asthma	HR# (95% CI)	New-onset asthma	No asthma	HR# (95% CI)
Quartile 1	13	393	1	9	81	2.55 (1.08–6.04)
Quartile 2	19	408	1.66 (0.82–3.39)	9	61	3.97 (1.67–9.41)
Quartile 3	18	395	1.80 (0.87–3.73)	7	65	3.77 (1.48–9.61)
Quartile 4	36	380	3.18 (1.66–6.08)	8	77	2.17 (0.88–5.34)
ptrend			<0.001¶			0.33¶
pinteraction			<0.05+			

Data are presented as n, unless otherwise stated. HR: hazard ratio; ptrend: p-value for trend; pinteraction: p-value for interaction. #: adjusted for race/ethnicity, lifetime wheeze and community with baseline strata for age and sex; ¶: trend tests conducted in stratified models; +: based on the Chi-squared statistic using the likelihood ratio test to compare a model with base terms to a model containing the interaction term.

aetiological role for inflammation in asthma pathogenesis. Further research is needed to determine whether pro-inflammatory environmental stressors may help to explain why we see the largest effects of *F*eNO in children without a parental history of asthma.

A body of evidence indicates that *F*eNO is elevated in allergic airway disease [4, 5] and studies have shown *F*eNO to be elevated in healthy atopic children [34]. We did not find evidence that the effect of *F*eNO depended on the child's allergy status. Children with and without a history of respiratory allergy showed similar patterns of increasing risk of new-onset asthma by increasing quartiles of *F*eNO.

While our results suggest that the relationship of *F*eNO with new-onset asthma may be independent of the allergic pathway, it is important to note that we used parent-report of hayfever or allergic rhinitis as a measure of children's respiratory allergy status, which may result in measurement error of true atopic status. However, measurement error is not likely to explain our entire findings as significant residual confounding by atopic status would likely occur only if the true associations between atopy and *F*eNO and between atopy and new onset asthma are both very strong (*e.g.* relative risks of 10.0) [35]. The strong relationships of self-reported measures of allergy with *F*eNO and asthma provide evidence that measurement error of atopic status is not likely to explain our findings.

The incidence rate of physician-diagnosed asthma in the present study (22.2 per 1,000 person-yrs) was higher than rates reported in earlier periods [36]. However, in recent decades, rates are more comparable, likely reflecting the increasing occurrence of childhood asthma [37, 38]. The incidence rate in the present study is consistent with earlier CHS cohorts of approximately the same age (17.8 per 1,000 person-yrs) [17]. By restricting our case definition to children without any history of wheezing, the incidence rate remained substantial (11.1 per 1,000 person-yrs).

A recognised limitation of our study is our reliance on self-report of physician-diagnosed asthma for defining eligibility and for case ascertainment. However, physician diagnosis of asthma has been widely accepted as a valid method of classifying asthma status in large epidemiological studies [39, 40]. In a subset of a previous CHS cohort, we independently verified self-reported physician-diagnosed asthma through a review of medical records and found that >95% of the children with a self-reported diagnosis had either a definite or probable asthma diagnosis noted on the medical record [41].

Another potential influence on asthma diagnosis is differential access to care and differences in medical practice. We found that adjustment for various indicators of socioeconomic status did not change our results, and we found that by restricting our analysis to cases using recent inhaled medication or by restricting to cases categorised as moderate or severe resulted in stronger risk estimates at each *F*eNO quartile; therefore, any bias that might arise from differences in medical practice is likely to attenuate the risk estimate toward the null. Children who reported use of inhaled medication after diagnosis could represent children with more bronchial inflammation before asthma onset and more severe asthma at diagnosis.

While this cohort was initially established when the participants were young (aged 5–6 yrs on average), we cannot definitively state that a new asthma diagnosis represents a true incident case. Misclassification of asthma status at cohort entry is not likely to explain our findings, as excluding cases reporting a diagnosis in the first year of follow-up did not substantially alter the relative risk estimates. To further limit the inclusion of possible undiagnosed asthma, we restricted our analysis to children without a history of wheeze or without wheeze in the twelve months prior to *F*eNO measurement. The results remained consistent with the highest risk of new-onset asthma occurring in children with the highest quartile of *F*eNO.

Conclusions

Our results suggest that *F*eNO is a marker of risk for the development of asthma especially among children without a

parental history of asthma. F_{ENO} may be valuable in developing primary prevention strategies.

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STATEMENT OF INTEREST

None declared.

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