ONLINE METHODS AND DATA SUPPLEMENT

Statistical Methods

In order to investigate the relationship of FeNO with new-onset asthma, we calculated incidence rates of new-onset asthma, conducted descriptive analyses of exposure variables with new-onset asthma, and explored a series of multivariate modeling approaches 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 FeNO. We have previously shown that FeNO varies with age.(1) Therefore, three age strata were defined with cutpoints at 8 and 9 years and these age strata were used to define FeNO quartiles for analyses.

To further investigate the association between FeNO and new-onset asthma with adjustment for potential confounders, we fitted Cox proportional hazards models with sex- and age-specific baseline hazards (with age defined as integer age at FeNO 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, family income, health insurance, family history of asthma, BMI, household pets or pests, humidifier use, lifetime secondhand tobacco smoke exposure, or *in utero* exposure to maternal smoking. Covariates were considered to be confounders if the hazard ratio changed by 10% after addition to a base model (that included adjustment for community and race/ethnicity). The final model was additionally adjusted for lifetime history of wheezing with stratified baseline hazards for sex and age. Heterogeneity of associations among subgroups was assessed by fitting models with appropriate interaction terms, and statistical significance was tested by partial likelihood ratio tests.(2)

Stratified analyses were performed in the presence of significant interaction (p-value< 0.05). The nature of the nonlinearity in FeNO effects was explored using splines, piecewise cubic polynomials that are joined smoothly at a number of breakpoints known as knots.(3)

Sensitivity analyses were conducted by limiting the asthma case definition to those (1) reporting a physician diagnosis more than one year after FeNO testing; (2) without a history of allergy; and (3) reporting use of inhaled medication within the previous twelve months. To explore the role of wheezing prior to the onset of asthma, we restricted to children without a history of wheezing and without wheezing in the twelve months prior to FeNO testing.

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

Statistical Methods for Fitting Spline-based Cox Regression Models

1. **Description of Figure 2**

The Cox regression model with one-knot quadratic regression spline term for the effect of FeNO as depicted in Figure 2 has the following form:

$$\lambda_{age,gender}(t) = \lambda_{0age,gender}(t) \cdot \exp\left(\beta_0 \cdot eNO + \beta_1 \cdot eNO^2 + \beta_2 \cdot (eNO - Knot_1)^{+^2} + \beta_3 \cdot X^T\right)$$

, where X denoted a set of the confounders and age and gender-specific baseline hazard was used. Note that the knot was selected based on a grid search that considered multiple knots and varying locations. The depicted smooth curve for the hazard ratio as function of FeNO is based on

$$\exp\left(\beta_0 \cdot eNO + \beta_1 \cdot eNO^2 + \beta_2 \cdot (eNO - Knot_1)^{+^2}\right)$$

, which describes the effect of baseline FeNO on the hazard of new-onset asthma, given other factors are the same. The entire cohort was used in fitting the above regression model. Figure 2 depicts the estimated overall hazard ratio for asthma incidence as a function of exhaled NO in the study cohort.

2. Description of Figure S1

The quadratic regression spline-based Cox regression model for Figure S1 has the following form:

$$\lambda_{age,gender}(t) = \lambda_{0age,gender}(t) \cdot \exp\left(\beta_0 \cdot eNO_{famhx=1} + \beta_1 \cdot eNO_{famhx=1}^2 + \beta_2 \cdot (eNO_{famhx=1} - Knot_{famhx=1})_+^2\right.$$
$$\left. + \beta_3 \cdot eNO_{famhx=0} + \beta_4 \cdot eNO_{famhx=0}^2 + \beta_5 \cdot \left(eNO_{famhx=0} - Knot_{famhx=0}\right)_+^2 + \beta_6 \cdot famhx + \beta_7 \cdot X^T\right)$$

, where X denotes a set of confounders, "famhx" represents an indicator of family history of asthma, incorporating age and gender-specific baseline hazard. The primary statistics of interest are family history of asthma-specific hazard ratios

$$\exp\left(\beta_0 \cdot eNO_{famhx=1} + \beta_1 \cdot eNO_{famhx=1}^2 + \beta_2 \cdot (eNO_{famhx=1} - Knot_{famhx=1})_+^2 + \beta_6\right)$$ for subjects with family history of asthma

and

$$\exp(\beta_3 \cdot eNO_{famhx=0} + \beta_4 \cdot eNO_{famhx=0}^2 + \beta_5 \cdot \left(eNO_{famhx=0} - Knot_{famhx=0}\right)_+^2)$$ for subjects without family history of asthma

, which describe the effect of baseline FeNO on the hazard new-onset asthma, given other factors are the same, in both subjects with family history of asthma and those without family history of asthma. Figure S1, therefore, describes the estimated family history of asthma specific hazard ratio for asthma incidence as a function of exhaled NO in the study cohort. The two curves in this plot are comparable because the main effect of family history of asthma was considered in the modeling framework.

REFERENCES

1.   Linn WS, Rappaport EB, Berhane KT, Bastain TM, Avol EL, Gilliland FD. Exhaled nitric oxide in a population-based study of southern california schoolchildren. *Respir Res* 2009;10:28.
2.   Cox. Regression models and life tables. *J Royal Statistical Soc* 1972;B:187-202.
3.   Hastie TJ, Tibshirani RJ. Generalized additive models. New York: Chapman and Hall; 1990.

Online Supplement Figure Legend

Figure S1. The Effect of Exhaled NO on New-Onset Asthma by Family History of Asthma

Table S1. FeNO Quartile Cutpoints by Age at Study Entry

	<8 years		8-9 years		>9 years	
	FeNO level (ppb)	N	FeNO level (ppb)	N	FeNO level (ppb)	N
Quartile 1	<7.1	175	<7.4	266	<7.7	111
Quartile 2	7.1 -9.8	174	7.4-10.2	266	7.7-10.4	111
Quartile 3	9.8-14.8	176	10.2-14.8	266	10.4-16.7	111
Quartile 4	>=14.8	174	>=14.8	266	>=16.7	110
		699		1064		443

Table S2. Incidence Rates of Asthma Among Study Participants

	Asthma Cases	Person-Years (pyrs)	Incidence Rate (per 1000 pyrs)	95% CI
All Children	129	5802.6	22.2	
Sex				
Female	69	3051.5	22.6	17.9-28.6
Male	60	2751.1	21.8	16.9-28.1
Race/Ethnicity				
White	47	2141.9	21.9	16.5-29.2
Hispanic	67	3150.1	21.3	16.7-27.0
African American	4	80.4	49.7	18.7-132.5
Asian	3	161.3	18.6	6.0-57.7
Other	8	262.6	30.5	15.2-60.9
Family History of Asthma				
No	86	4388.3	19.6	15.9-24.2
Yes	34	869.0	39.1	28.0-54.8
Age at Study Entry				
<8 years	40	1863.9	21.5	15.7-29.3
8 to 9 years	58	2818.5	20.6	15.9-26.6
>9 years	31	1120.2	27.7	19.5-39.4
Age-specific Quartile of FeNO				
FeNO quartile 1	24	1501.3	16.0	10.7-23.8
FeNO quartile 2	30	1438.4	20.9	14.6-29.8
FeNO quartile 3	30	1454.5	20.6	14.4-29.5
FeNO quartile 4	45	1408.3	32.0	23.9-42.8
Children Without History of Wheezing	48	4310.2	11.1	
Sex				
Female	29	2289.6	12.7	8.8-18.2
Male	19	2020.6	9.4	6.0-14.7
Race/Ethnicity				
White	18	1517.3	11.9	7.5-18.8
Hispanic	23	2412.9	9.5	6.3-14.3
African American	4	57.6	69.5	26.1-185.1

Asian	2	135.3	14.8	3.7-59.1
Other	1	180.9	5.5	0.8-39.3
Family History of Asthma				
No	33	3356.1	9.8	7.0-13.8
Yes	12	532.8	22.5	12.8-39.7
Age at Study Entry				
<8 years	13	1365.8	9.5	5.5-16.4
8 to 9 years	25	2117.4	11.8	8.0-17.5
>9 years	10	827.1	12.1	6.5-22.5
Age-specific Quartile of FeNO				
FeNO quartile 1	8	1065.4	7.5	3.8-15.0
FeNO quartile 2	16	1119.5	14.3	8.8-23.3
FeNO quartile 3	8	1144.0	7.0	3.5-14.0
FeNO quartile 4	16	981.3	16.3	10.0-26.6

Table S3. Exhaled Nitric Oxide (FeNO) and Risk of New-Onset Asthma: Restricted Case Definitions to those Reporting Recent Medication Use in the Diagnosis Year†

Age-Specific Quartiles of FeNO at Baseline	Restricted to Cases Reporting Recent Inhaled Medication Use (N=2133; 56 cases)		Restricted to Cases Reporting Recent Rescue Medication Use Only (N=2108; 31 cases)		Restricted to Cases Reporting Recent Controller Medication Use Only (N=2084; 7 cases)		Restricted to Cases Reporting Recent Controller and Rescue Medication Use (N=2101; 24 cases)	
	HR*	95% CI	HR*	95% CI	HR*	95% CI	HR*	95% CI
Quartile 1	1		1		1		1	
Quartile 2	3.01	1.14-7.89	4.49	0.92-21.87	3.65	0.33-39.81	1.59	0.35-7.24
Quartile 3	3.57	1.35-9.44	5.97	1.24-28.89	2.97	0.17-50.45	2.18	0.50-9.47
Quartile 4	4.29	1.70-10.80	4.22	1.73-35.71	2.44	0.20-29.27	4.08	1.11-15.07
	$p_{trend} < 0.005$		$p_{trend} < 0.005$		$p_{trend} = $ n.s.		$p_{trend} < 0.05$	

*HR=Hazard ratio, adjusted for race/ethnicity, community, and lifetime wheeze and stratified by integer age and sex

†Recent inhaled medication use is defined as any use in the previous 12 months, reported on the diagnosis year follow-up questionnaire.