Contributions of asthma, rhinitis and IgE to exhaled nitric oxide in adolescents

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ABSTRACT Exhaled nitric oxide fraction (FeNO) is an indicator of allergic airway inflammation. However, it is unknown how asthma, allergic rhinitis (AR) and allergic sensitisation relate to FeNO, particularly among adolescents and in overlapping conditions. We sought to determine the associations between asthma, AR, and aeroallergen immunoglobulin (Ig)E and FeNO in adolescents.

We measured FeNO among 929 adolescents (aged 11–16 years) in Project Viva, an unselected prebirth cohort in Massachusetts, USA. We defined asthma as ever asthma physician diagnosis plus wheezing in the past year or taking asthma medications in the past month, AR as a physician diagnosis of hay fever or AR, and aeroallergen IgE as any IgE >0.35 IU·mL−1 among 592 participants who provided blood samples. We examined associations of asthma, AR and IgE with percent difference in FeNO in linear regression models adjusted for sex, race/ethnicity, age and height, maternal education and smoking during pregnancy, and household/neighborhood demographics.

Asthma (14%) was associated with 97% higher FeNO (95% CI 70–128%), AR (21%) with 45% higher FeNO (95% CI 28–65%), and aeroallergen IgE (58%) with 102% higher FeNO (95% CI 80–126%) compared to those without each condition, respectively. In the absence of asthma or AR, aeroallergen IgE was associated with 75% higher FeNO (95% CI 52–101), while asthma and AR were not associated with FeNO in the absence of IgE.

The link between asthma and AR with FeNO is limited to those with IgE-mediated phenotypes. FeNO may be elevated in those with allergic sensitisation alone, even in the absence of asthma or AR.

Cite this article as: Flashner BM, Rifas-Shiman SL, Oken E, et al. Contributions of asthma, rhinitis and IgE to exhaled nitric oxide in adolescents. ERJ Open Res 2021; 7: 00945-2020 [https://doi.org/10.1183/23120541.00945-2020].
Introduction

Exhaled nitric oxide fraction ($F_{eNO}$) is an indicator of allergic airway inflammation and can aid in the diagnosis of allergic asthma [1]. The influence of concurrent allergic rhinitis (AR) and allergic sensitisation to environmental allergens on $F_{eNO}$ measurements is not well-known in adolescents. How each of these (asthma, AR and aeroallergen immunoglobulin (IgE)) relate to $F_{eNO}$ is important not only for interpretation of $F_{eNO}$ in clinical use but also for a greater understanding of the factors that lead to elevated $F_{eNO}$. We sought to understand the associations of asthma, AR, and aeroallergen IgE with $F_{eNO}$ in an adolescent population.

Asthma and AR are common and contribute to significant morbidity, with poorer quality of life, missed days of school, healthcare costs and emergency room visits for asthma exacerbations, resulting from difficulty sleeping, fatigue, and changes in mood and cognition [2]. AR and asthma share epidemiological overlap. AR occurs in >75% of patients with asthma, and asthma is seen in up to 40% of those with AR [3]. In those with both AR and asthma, AR may present first and there is a risk factor for subsequent development of asthma [4–7].

Asthma and AR are each known to be associated with elevated $F_{eNO}$ in both adults and children. Nitric oxide (NO) is an intercellular messenger known to mediate a variety of processes, including immune function and inflammation [8]. In the lung, NO acts as a signalling molecule in bronchial and vascular dilatation, ciliary kinesis, and neurotransmission in the non-adrenergic and non-cholinergic systems [9]. In allergic inflammation, T-helper-2 cells, type 2 innate lymphoid cells, mast cells and eosinophils produce cytokines, like interleukin (IL)-4 and IL-13, with downstream effects including the activation of inducible NO synthase (iNOS) and thus elevated NO levels. Therefore, $F_{eNO}$ is considered a marker of type 2 airway inflammation, which occurs with AR in the upper airway or allergic asthma in the lower airway [9–13]. Elevation of $F_{eNO}$ may indicate not only allergic asthma or AR but also elevations in circulating IgE against environmental allergens. In previous work in this same Project Viva cohort, an unselected pre-birth cohort in Massachusetts, USA, we have found differences in the nasal epigenome in association with asthma, AR, aeroallergen IgE and $F_{eNO}$ that are annotated with genes implicated in type 2 inflammatory responses [14]. Currently, the American Thoracic Society recommends $F_{eNO}$ in the diagnosis of eosinophilic airway inflammation, and suggests that it may be used to support the diagnosis of asthma where the diagnosis is less clear [1]. $F_{eNO}$, however, may also be elevated in AR or allergic sensitisation to environmental allergens [15, 16]. Therefore, we sought to elucidate the contributors to $F_{eNO}$ and supplement our understanding of its diagnostic utility. In this study, we examined the relationships of asthma, AR, and aeroallergen IgE with $F_{eNO}$ in an adolescent population in Project Viva, a cohort not selected on the basis of asthma or allergy.

Materials and methods

Study design and participants

Between 1999 and 2002 we recruited women in early pregnancy into Project Viva from eight obstetric offices of Atrius Harvard Vanguard Medical Associates, a multi-specialty group practice in eastern Massachusetts. Exclusion criteria included multiple gestation, inability to answer questions in English, and gestational age ⩾ 22 weeks at recruitment. Details of recruitment and retention are available elsewhere [17]. Of the 2128 infants, we included in this analysis those participants with $F_{eNO}$ measurements at an in-person visit in early adolescence, which totalled 929. Compared with the 929 included participants, the 1199 excluded participants were less likely to have college-educated mothers (59% versus 71%) and more likely to have mothers who smoked during pregnancy (15% versus 9%). However, maternal age at enrolment, gestational age at delivery, and sex and race/ethnicity were similar. Those 1199 were excluded due to either missing $F_{eNO}$ or no early adolescent visit. The median adolescent age was 12.9 years with a range of 11.9–16.6 years; there were no participants older than this due to cohort inception dates.

Asthma and AR

We defined current asthma as a maternal report of ever asthma diagnosis plus wheeze symptoms in the past 12 months or use of asthma medications in the past month, reported on an early teen questionnaire in keeping with the Study of Asthma and Allergies in Childhood questionnaire [18]. We used as a comparison group with no asthma diagnosis, no wheezing and no use of asthma medications or wheezing in the past 12 months. Participants were defined as having AR if they reported ever receiving a physician diagnosis of hay fever or AR. Both current asthma and AR were reported by parental questionnaire at the early teen follow-up visit.

Aeroallergen IgE

Trained research phlebotomists collected blood from participants at the early teen visit, which we centrifuged and stored at −80C. We measured plasma IgE against Dermatophagoides farina (dust mite),
We examined the associations of asthma, AR, and aeroallergen IgE with $eNO >35$ ppb based on American Thoracic Society guidelines [1] using multinomial logistic regression models.

Without asthma, those with AR had a 45% higher and those with aeroallergen IgE had 102% higher $eNO$. Perennial IgE was associated with 119% higher $eNO$. To examine associations of overlapping conditions of asthma, AR and aeroallergen IgE, we derived an eight-category exposure based on the combination of these three exposures. We ran linear regression models for the associations of seasonal and perennial IgE with $eNO$.

Measurement of $F_{eNO}$

Exhaled NO levels were measured twice for each participant with a portable electrochemical device (NIOX MINO; Aerocrine AB); this has been validated by chemiluminescence technology, with an accuracy of ±5 ppb [21]. Prior to each measurement, participants breathed in through an NO scrubbing filter and exhaled into room air twice. This was done in keeping with prior studies using $F_{eNO}$; the ambient air NO was not measured [22–24]. On the third breath, participants inhaled through the filter and exhaled into the $F_{eNO}$ analyser. The last 3 s of the exhalation were utilised for $F_{eNO}$ measurement; this ensures lower rather than upper airway measurement. Nose clips were not used.

**Statistical analysis**

Potential confounders were selected *a priori*, based on known or suspected associations with asthma or allergy. Model 1 adjusted for sex and age and height at the early teen visit. Model 2 additionally adjusted for race/ethnicity, maternal education and smoking during pregnancy, median value of owner-occupied housing and education (% with a bachelor degree) based on census tract of home residence at time of mid-childhood visit from 2000, and household income and any smokers at home at the early teen visit. We additionally adjusted for body mass index in model 3.

We averaged the two $F_{eNO}$ measurements and included the log-transformed value as a continuous outcome in linear regression models. We present effect estimates as % change (95% CI) for $F_{eNO}$, calculated as $(\exp(\beta)-1)\times100$. In secondary analyses, we examined $F_{eNO}$ in categories ($<20$, $20–\leq35$ and $>35$ ppb) based on American Thoracic Society guidelines [1] using multinomial logistic regression models. We examined the associations of asthma, AR, and aeroallergen IgE with $F_{eNO}$ using separate linear and logistic regression models. We repeated linear regression models for the associations of seasonal and perennial IgE with $F_{eNO}$.

To examine associations of overlapping conditions of asthma, AR and aeroallergen IgE, we derived an eight-category exposure based on the combination of these three exposures. We ran linear regression models with this eight-category exposure and used no aeroallergen IgE, no AR, no asthma as the reference category. We performed all analyses using SAS 9.4 (SAS Institute).

**Results**

**Study population**

The characteristics of the 929 study participants included in any analysis and their mothers are shown in table 1. Reported smoking in the home was rare (12%) and mean reported annual household income was $109,000. Children were predominantly white (64%) and their average age was 13 years. Mean±SD $F_{eNO}$ was 26±27 ppb and 19% of study participants had exhaled NO levels above the upper limit of normal for this age group (35 ppb) [1].

Overall, 115 (14%) out of 797 participants reported asthma while 179 (21%) out of 869 reported AR. Aeroallergen IgE $>0.35$ IU·mL$^{-1}$ was detected in 345 (58%) out of 592 of those providing blood samples with IgE results. Mean±SD $F_{eNO}$ in those with asthma was 48.4±43.0 ppb compared to 21.8±20.4 ppb in those without asthma. Mean±SD $F_{eNO}$ in those with AR was 35.7±32.8 ppb compared to 23.5±24.9 ppb without AR. Participants with aeroallergen IgE had mean±SD $F_{eNO}$ 36.2±34.2 ppb compared to 14.8±9.5 ppb in those without IgE.

Associations of each condition with $F_{eNO}$ are shown in table 2, where model 1 is parsimoniously adjusted and model 2 is fully adjusted, with similar results. Asthma was common in those with $F_{eNO} >35$ ppb; 35% of teens with $F_{eNO} >35$ ppb had asthma versus 7% among participants with $F_{eNO} <20$ ppb and 18% among participants with $F_{eNO} 20–\leq35$ ppb. Of the three conditions, the presence of aeroallergen IgE and asthma diagnosis each had a similar association with $F_{eNO}$, while the magnitude of the association was half as great for AR. Specifically, those with asthma had a 97% higher $F_{eNO}$ (95% CI 70–128%) compared to those without asthma. Those with AR had a 45% higher $F_{eNO}$ (95% CI 28–65%) compared to those without AR, and those with aeroallergen IgE had 102% higher $F_{eNO}$ (95% CI 80–126%) compared to those without aeroallergen IgE. Perennial IgE was associated with 119% higher $F_{eNO}$ (95% CI 97–144) while seasonal IgE...
was associated with 71% higher $F_{eNO}$ (95% CI 52–93). Similar results were seen in logistic regression analyses for the clinical thresholds of $F_{eNO}$. Asthma was associated with eight times higher odds (95% CI 5–14) of $F_{eNO}$ >35 ppb, AR was associated with three times higher odds (95% CI 2–5) of $F_{eNO}$ >35 ppb and aeroallergen IgE was associated with 28 times higher odds (95% CI 12–63) of $F_{eNO}$ >35 ppb compared to those without each condition (table 2).

We found similar associations when stratified by sex (table S2). Since steroids can affect $F_{eNO}$, we ran a sensitivity analysis, excluding participants who used oral or inhaled corticosteroids within 72 h of $F_{eNO}$ testing (n=22) and found similar associations between asthma, AR and aeroallergen IgE with higher $F_{eNO}$.

### TABLE 1 Participant characteristics (N=929)

| Subjects n                                      | 929 |
|-----------------------------------------------|-----|
| **Child**                                     |     |
| Sex                                           |     |
| Male                                          | 466 (50) |
| Female                                        | 463 (50) |
| Race/ethnicity                                |     |
| Black                                         | 154 (17) |
| Hispanic                                      | 40 (4) |
| Asian                                         | 28 (3) |
| White                                         | 593 (64) |
| Other or >1 race/ethnicity                    | 113 (12) |
| **Early teen visit**                          |     |
| Age years                                     | 13±1 |
| Age at visit years                            |     |
| 11.9 to <13                                   | 505 (54.4) |
| 13.0 to <15.0                                 | 374 (40.3) |
| 15.0 to 16.6                                  | 50 (5.4) |
| Height cm                                     | 160±9 |
| BMI percentile category %                     |     |
| <5th                                          | 30 (3) |
| 5–<85th                                       | 634 (68) |
| >85th                                         | 262 (28) |
| Exhaled nitric oxide ppb                      |     |
| <20 ppb                                       | 553 (60) |
| 20–≤35 ppb                                    | 196 (21) |
| >35 ppb                                       | 180 (19) |
| Current asthma                                |     |
| No                                            | 682 (86) |
| Yes                                           | 115 (14) |
| Ever allergic rhinitis                        |     |
| No                                            | 690 (79) |
| Yes                                           | 179 (21) |
| Any aeroallergen IgE >0.35 kU·L$^{-1}$         |     |
| No                                            | 247 (42) |
| Yes                                           | 345 (58) |
| Annual household income at early teen visit $1000 |       |
| No                                            | 109±44 |
| Yes                                           | 114 (12) |
| Any smokers at home at early teen visit       |     |
| No                                            | 634 (68) |
| Yes                                           | 285 (31) |
| Mother/family                                  |     |
| Pregnancy smoking status                      |     |
| Never                                         | 653 (71) |
| Former                                        | 188 (20) |
| During pregnancy                              | 85 (9) |
| College graduate                              |     |
| No                                            | 264 (29) |
| Yes                                           | 662 (71) |
| Median value owner-occupied housing $1000#    |     |
| No                                            | 263±149 |
| Yes                                           | 42±20 |

Data are presented as n (%) or mean±SD, unless otherwise stated. BMI: body mass index; Ig: immunoglobulin. #: based on census tract, mid-childhood.
Finally, we adjusted for body mass index and found similar associations with asthma, AR and aeroallergen IgE with \( \text{FeNO} \) in both linear and logistic regression models (table S3).

### Overlapping associations of allergy, asthma and aeroallergen IgE with \( \text{FeNO} \)

To examine associations of overlapping conditions of asthma, AR and aeroallergen IgE, we derived an eight-category exposure based on the combination of these three exposures among 476 participants with non-missing values for the three exposures. The reference category was no asthma, no AR and no aeroallergen IgE (figure 1, table S1). We found that, in the absence of asthma or AR, aeroallergen IgE alone (n=173, 36%) was associated with 75% higher \( \text{FeNO} \) (95% CI 52–101%). Asthma in the absence of AR and aeroallergen IgE (n=10, 2%), AR in the absence of asthma and aeroallergen IgE (n=14, 3%), and asthma and AR in the absence of aeroallergen IgE (n=6, 1%) were uncommon and not associated with

![FIGURE 1](https://doi.org/10.1183/23120541.00945-2020)  
**FIGURE 1** Percent difference in exhaled nitric oxide fraction (\( \text{FeNO} \)) relative to reference group a) without detectable aeroallergen immunoglobulin (IgE) and b) with aeroallergen IgE. AR: allergic rhinitis.
The presence of all three exposures (asthma, AR and aeroallergen IgE \(n=32, 7\%\)) was associated with 272% higher \(F_{ENO}\) (95% CI 189–379%) compared to having none of these (figure 1).

**Discussion**

In this study of adolescents, we found, as expected, that asthma and allergy were each associated with higher \(F_{ENO}\). However, this association was absent among those without detectable aeroallergen IgE. Additionally, aeroallergen IgE was associated with elevated \(F_{ENO}\) even in the absence of asthma or AR.

The association between asthma and elevated \(F_{ENO}\) among adolescents in this study is consistent with the well-established connection between allergic asthma and airway inflammation as measured by \(F_{ENO}\). Exhaled NO >35 ppb in children strongly supports the diagnosis of asthma and can be used as a diagnostic tool [1]. \(F_{ENO}\) is a marker of an allergic asthma phenotype, characterised by elevations in type 2 cytokines IL-4, IL-5 and IL-13 and eosinophils in sputum, serum, and bronchial biopsy [25–27].

However, we found that this association between asthma and increased \(F_{ENO}\) was seen only when aeroallergen IgE was detected. In the absence of aeroallergen IgE, the association between asthma and \(F_{ENO}\) was lost. Our results suggest that asthma is only associated with \(F_{ENO}\) in the presence of allergic sensitisation, and that \(F_{ENO}\) is more likely to indicate the presence of aeroallergen IgE. We found that \(F_{ENO}\) >35 ppb had a positive predictive value of only 35% for the diagnosis of asthma, compared to a positive predictive value of 94% for the presence of aeroallergen IgE. Our findings contrast with a study of 1156 children in France, which found that non-atopic asthmatics had higher \(F_{ENO}\) than non-atopic children without asthma: average \(F_{ENO}\) 13.4 ppb in non-atopic children with asthma versus 10.6 ppb in non-atopic children without asthma [28]. However, our findings are consistent with other birth cohorts, including one from the Netherlands, which observed higher \(F_{ENO}\) only among young adults with atopic asthma, defined as asthma plus allergen sensitisation with positive serum IgE against 10 common allergens, but not non-atopic asthma [29]. The Isle of Wight birth cohort in England, UK, similarly found that atopy (measured by skin-prick testing) was associated with higher \(F_{ENO}\) while the level of \(F_{ENO}\) did not differ between non-atopic teens with and without asthma [30]. The authors concluded, as we do, that \(F_{ENO}\) is a biomarker for atopy rather than asthma.

Our study also confirmed the association between AR and higher \(F_{ENO}\) among adolescents, which has been well established among adults and children [31–35]. Similar to our findings in asthma, we found that this association between AR and \(F_{ENO}\) was observed only in the presence of aeroallergen IgE. Other studies have also found that those with atopy and rhinitis have higher \(F_{ENO}\) than those with rhinitis but no atopy [28, 36, 37]. This same large cohort of 1156 children in France found that non-asthmatic atopic participants (as determined by skin-prick test to common allergens) with rhinitis had significantly higher \(F_{ENO}\) than non-atopic participants with rhinitis (20.7±13 versus 12.5±6.4 ppb) [28]. Likewise, in an adult population examining atopic participants identified by skin-prick testing, GRATZIOU _et al._ [37] found that \(F_{ENO}\) was significantly higher in atopic rhinitis than in those with non-atopic rhinitis (13.3±1.3 versus 5.8±1.2 ppb).

One explanation of our findings is that asthma in the setting of low aeroallergen IgE is mediated by a non-allergic inflammatory pathway. This distinct non-allergic asthma phenotype represents a subset of asthmatics that may have more severe and more difficult to control asthma [38, 39]. Rather than allergic, type 2-mediated pathways, these non-atopic asthmatics may have neutrophilic airway inflammation [40]. This may be determined, in part, by genetic factors, and those with allergic and non-allergic asthma have distinct HLA haplotypes [41]. Environmental exposures such as particulate air pollution or childhood viral infections may also contribute to non-allergic asthma, in a process mediated through neutrophilic inflammation [42]. A similar neutrophilic process may be occurring in our participants with asthma or AR and no significant serum IgE.

We found that aeroallergen IgE is associated with elevated \(F_{ENO}\) even in the absence of asthma or AR. A few studies have similarly found that atopy is associated with \(F_{ENO}\) regardless of symptoms. For example, both _CHOI et al._ [36] and _FRANKLIN et al._ [43] examined children with asthma and atopy and found that atopy increases \(F_{ENO}\) regardless of asthma diagnosis. This has been similarly observed among Pacific Islander adults, where positive skin-prick testing to house dust mite was associated with higher exhaled and nasal NO even in the absence of asthma symptoms [44]. In a study comparing asthmatic and healthy children, _BARRETO et al._ [45] found that even in those without respiratory symptoms, atopy (defined by skin-prick testing) and peripheral eosinophil count were associated with significantly higher \(F_{ENO}\) compared to those without atopy or eosinophilia. In a population of school children, _VAN AMSTERDAM et al._ [46] similarly found that allergic sensitisation was associated with higher \(F_{ENO}\) even in those without wheeze, although the association was significantly augmented in those with wheeze.

Our findings suggest that IgE is an important factor in the elevated \(F_{ENO}\) observed in asthma and AR in adolescents. While the exact mechanism by which IgE may influence \(F_{ENO}\) is not entirely clear, the
pathophysiology by which each are elevated in asthma and AR has been previously explored. IgE may increase \( F_{\text{ENO}} \) along with inflammatory cytokines inducing iNOS. In immediate hypersensitivity reactions, IgE cross-links to the FceRI receptor on mast cells, resulting in mast cell degranulation, inflammatory cytokine release, and activation of inflammatory cells at sites sensitive to the inciting allergy. Local IgE-mediated mechanisms may explain the localised reactions in skin, nasal turbinates, airways, and gut in eczema, rhinitis, asthma, and food allergy, respectively. In allergic asthma and AR specifically, the inflammatory cytokines that occur as a result of IgE-FceRI cross-linking may induce iNOS, resulting in the increased \( F_{\text{ENO}} \) observed in AR and asthma [47, 48]. The upregulated eosinophils in type 2 inflammation of asthma and AR may also exert direct oxidative damage and promote the continued release of inflammatory cytokines that activate iNOS, increasing NO in exhaled air [28, 45, 49].

Our study is one of the largest studies conducted in a well-characterised adolescent age group that examines the associations of asthma, allergy and IgE with airway inflammation as measured by \( F_{\text{ENO}} \). However, we acknowledge limitations to this study. Specifically, this is a cross sectional study evaluating \( F_{\text{ENO}} \) asthma and AR at a single time-point in adolescence. Asthma and AR vary widely in symptoms, severity and control, and so associations may change depending on different time-points in the disease trajectory. Additionally, steroid use may reduce \( F_{\text{ENO}} \), although we had similar findings when excluding participants with any steroid use within 72 h of testing. In addition, there were very few participants who had asthma or AR but no aeroallergen IgE. This may represent the fact that the majority of those with asthma or AR in this study had an allergic asthma phenotype. There were, however, a large portion of our participants (36%) who did not have asthma or AR but who did have aeroallergen IgE. Finally, we did not include those with previous or non-active asthma for those without wheeze in the last 12 months. Similarly, we did not include IgE positivity to non-tested allergens such as food-specific IgE. Future work may include these other allergens. We would also explore the comparison of \( F_{\text{ENO}} \) with blood eosinophils as a more widely available biomarker than \( F_{\text{ENO}} \).

Our study confirms the expected pattern of \( F_{\text{ENO}} \) elevation among adolescents with asthma and AR and implicates IgE-mediated pathways in the association between asthma, AR and \( F_{\text{ENO}} \). Our findings suggest that the presence of aeroallergen IgE was critical to the \( F_{\text{ENO}} \) elevations observed in those with asthma or AR. Without aeroallergen IgE, that association was lost. Aeroallergen IgE can be considered a marker for elevated \( F_{\text{ENO}} \), since \( F_{\text{ENO}} \) was detected whenever aeroallergen IgE was present, regardless of the presence of clinical disease. Furthermore, even if \( F_{\text{ENO}} \) is elevated, it does not necessarily indicate asthma or AR, and patients with asthma and no aeroallergen IgE may not have an elevated \( F_{\text{ENO}} \). Therefore, this study places \( F_{\text{ENO}} \) into perspective as a marker predominantly for sensitisation rather than for clinical disease, and further raises the need to identify alternative markers for airway inflammation in those children and adolescents with non-IgE-mediated asthma or AR.

Author contributions: B.M. Flashner drafted the manuscript. M.B. Rice contributed to the drafting and revision of the manuscript. S.L. Rifas-Shiman conducted the analyses. E. Oken, C.A. Camargo Jr, T.A.E. Platts-Mills, L. Workman, A.A. Litonjua and D.R. Gold contributed to the revision of the manuscript.

Support statement: This work was supported by the US National Institutes of Health (K23ES026204, R01 HD034568, R01AI02960, UH3OD023286), the American Thoracic Society Foundation and the American Lung Association. Funding information for this article has been deposited with the Crossref Funder Registry.

Conflict of interest: B.M. Flashner has nothing to disclose. S.L. Rifas-Shiman reports grants from the US National Institutes of Health during the conduct of the study. E. Oken reports grants from the US National Institutes of Health during the conduct of the study. C.A. Camargo Jr has nothing to disclose. T.A.E. Platts-Mills has nothing to disclose. L. Workman has nothing to disclose. A.A. Litonjua has nothing to disclose. D.R. Gold reports grants from the US National Institutes of Health during the conduct of the study. M.B. Rice reports grants from the US National Institutes of Health during the conduct of the study.

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