Origins of asthma in childhood
Savenije, Olga Elisabeth Maria

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Chapter 5

Childhood wheezing phenotypes and FeNO in atopic children at age 8

RJP van der Valk, D Caudri, O Savenije, GH Koppelman, HA Smit, AH Wijga, DS Postma, M Kerkhof, B Brunekreef, JC de Jongste

Abstract

Background
Fractional exhaled Nitric Oxide (FeNO) is a surrogate biomarker of the degree of eosinophilic airway inflammation. Using longitudinal latent class analysis, five wheezing phenotypes have been identified, characterized by different ages of onset and prognosis.

Objectives
To assess FeNO measured at 4 and 8 years in children with different phenotypes of wheeze and atopy.

Methods
Children participated in the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) study, a prospective birth cohort in the Netherlands. Respiratory health was assessed yearly by questionnaires until the age of 8 years; these data were used to identify five wheezing phenotypes. Associations between FeNO and wheezing phenotypes were investigated using weighted linear regression.

Results
Data on wheezing phenotypes and FeNO at 4 and 8 years were available in 588 and 973 children respectively. Compared with the phenotype of never and transient wheeze, FeNO at 4 years was higher in intermediate onset and persistent wheeze. FeNO at 8 years of age differed significantly between all phenotypes, with highest FeNO values for persistent, intermediate onset, and late onset wheeze. Rise in FeNO from 4 to 8 years in intermediate and late onset wheezers was significantly higher compared to FeNO rise in never and transient wheezers. Stratified analyses showed that the increase in FeNO in persistent, intermediate, and late onset wheeze was only present in children with allergic sensitization at 8 years.

Conclusions and Clinical Relevance
The FeNO measured at 8 years was associated with specific wheezing phenotypes, only among atopic children.
Introduction

The fraction of nitric oxide in exhaled air (FeNO) is a non-invasive surrogate biomarker of the degree of eosinophilic airway inflammation with excellent reproducibility in childhood. Recent studies have shown that FeNO can be used both in large population-based studies and in clinical asthma management studies. Elevated FeNO was found in children and adults with asthma and atopy, overlapping with the distribution in normals. We previously reported on FeNO in 4-year-old children from the The Prevention and Incidence of Asthma and Mite Allergy (PIAMA) cohort, and found no association with classic wheezing phenotypes as described by Martinez in preschool children. However, FeNO may be influenced by atopy, which can develop later in life. PIAMA birth cohort study provided the opportunity to study FeNO in relation to phenotypes of wheeze in a large group of children recruited from the general population. One of the special features of PIAMA is the yearly respiratory health assessment, which can be used to define phenotypes of wheeze. Recently, phenotypes of wheeze were identified by Longitudinal Latent Class Analysis (LLCA) in the Avon Longitudinal Study of Parents and Children (ALSPAC) study, and these phenotypes were differently associated with atopy and lung function. This analysis was repeated in the PIAMA study and resulted in comparable phenotypes with similar associations with doctor-diagnosed asthma, inhaled corticosteroid use, sensitization to common allergens, forced expiratory volume in 1 second, and bronchial responsiveness. FeNO has not been studied in relation to phenotypes identified using this novel approach. We hypothesized that the different wheezing phenotypes are characterized by differences in eosinophilic inflammation, which would be reflected by differences and change in FeNO measured at the age of 4 and 8 years. Because atopy is an important determinant of FeNO, we stratified our analysis for atopy.

Materials and methods

Study design

The Prevention and Incidence of Asthma and Mite Allergy study is a prospective birth cohort study in the Netherlands. Recruitment took place in 1996–97 through prenatal clinics; 7862 pregnant women were invited to participate, 4146 (53%) agreed and gave informed consent. Children were labelled as high risk (n = 1327) and low risk (n = 2819), based on the atopic status of the mother. Respiratory health and asthma symptoms of the children were assessed yearly by questionnaires, partly based on the International Study of Asthma and Allergies in Childhood (ISAAC) core questionnaires, along with data on demographics and a wide range of asthma
risk factors. All high-risk children and a subgroup of low-risk children were invited for FeNO measurement at the age of 4 years (n = 1808) and 8 years (n = 1554). A detailed description of the PIAMA study design was previously published. The study protocol was approved by the medical ethics committees of the participating medical centres (Groningen: M 4.019912, Rotterdam: MEC 2004-152, and Utrecht: CCMO P04.0071C 04-101/K).

**Study population**

At the age of 4 years all high-risk (n = 1173) and a random sample of low-risk children (n = 635) were invited for a medical examination, including offline FeNO measurement. Of those 1808 children 1269 attended the examination, and an exhaled air sample was obtained in 939 children. Offline FeNO measurements of sufficient quality were obtained in 595 children (63%) at age 4. At 8 years also all high-risk children still in follow-up (n = 988) and a similar, random sample of low-risk children (n = 566) were invited for a hospital-based medical examination including online FeNO measurement. Of these 1554 children 1129 (73%) gave informed consent and attended the examination. In 39 children a FeNO measurement could not be performed due to device failure. Of the remaining 1090 children at least one successful FeNO measurement was obtained in 976 children (90%). The other 114 children were unable to exhale at a constant flow during FeNO measurement. A detailed flow chart of the study population with complete data on confounders, wheezing phenotypes, and FeNO at 4 years (n = 588) and 8 years (n = 973) is presented in Figure 1.

**Measurements**

The FeNO in 4-year-old children was measured offline by the balloon method, according to European Respiratory Society (ERS)/American Thoracic Society guidelines (ATS). FeNO in 8-year-old children was measured online using the NIOX chemiluminescence analyser (Aerocrine AB, Solna, Sweden) according to ERS and ATS guidelines. We previously found good agreement between these online and offline FeNO measurements. At 8 years blood was drawn to assess sensitization to airborne allergens, defined as specific IgE of 0.70 IU/mL for at least one of the following allergens: house dust mite (Dermatophagoides pteronyssinus), cats, dogs, grass pollen (Dactylis glomerata), birch, and Alternaria alternata.
Figure 1. Flow chart of study population at 4 and 8 years.
* These 4146 consisted of 1327 atopic (32%) and 2819 non-atopic mothers (68%), which is a good reflection of the general Dutch population.
Phenotypes of wheeze

Longitudinal latent class analysis was used by Savenije et al. to define wheezing phenotypes in PIAMA in early childhood, as originally published by Henderson et al.\textsuperscript{13} Wheezing phenotypes were previously defined in children with at least data on wheezing at two or more occasions, and in a subgroup of children with complete data on wheezing at every age from 1 to 8 years.\textsuperscript{14} There were no major differences between these two analyses. In the current analysis phenotypes derived in children with at least two wheezing observations were used, to minimize the risk of selection bias. Five wheezing phenotypes were identified in the first 8 years of life: never/infrequent wheeze (73.2%), transient early wheeze (17.3%), intermediate onset wheeze (3.4%), persistent wheeze (4.3%), and late onset wheeze (1.8%). These phenotypes were comparable with those identified in the ALSPAC cohort.\textsuperscript{14} The five phenotypes are graphically depicted in Figure 2.

![Figure 2. Probability of wheeze at each time point from birth to age 8 years for each wheezing phenotype in the prevention and incidence of asthma and mite allergy (N = 3,789). The prevalences of the phenotypes are shown next to the phenotypes in the legend. Figure adjusted from Savenije & Granell et al., with copyright permission.\textsuperscript{14}](image)

**Figure 2.** Probability of wheeze at each time point from birth to age 8 years for each wheezing phenotype in the prevention and incidence of asthma and mite allergy (N = 3,789). The prevalences of the phenotypes are shown next to the phenotypes in the legend. Figure adjusted from Savenije & Granell et al., with copyright permission.\textsuperscript{14}

Statistical analysis

All analyses were carried out in SAS 9.1 (SAS Institute, Inc., Cary, NC, USA). The associations between FeNO at 4 and 8 years and phenotypes of wheeze were investigated with weighted linear regression models (SAS PROC GENMOD). FeNO data were log-transformed, to achieve a normal distribution for linear regression analyses and back-transformations were used to calculate geometric mean FeNO for the phenotypes of wheeze. Due to the stratified study design, all analyses were performed for the total study population as well as for the high-risk and low-risk
children separately. The analyses were also stratified for allergic sensitization at 8 years, because specific IgE is an important determinant of FeNO. Individual membership probabilities (each child gets a probability to belong to each phenotype) derived from LLCA were used as weight factors in the linear regression models to minimize the risk of misclassification of the wheezing phenotypes. Gender, recent symptoms of cold, steroid use, study region, education of the mother, and exposure to environmental tobacco smoke were considered as potential confounders. Confounders were included in the models based on their association with wheezing phenotypes, or if they changed the effect estimate by more than 10%.

**Results**

**General characteristics of the study population**

Baseline characteristics at 4 and 8 years are given in Table I. Due to the study design, high-risk children were overrepresented in comparison with the total PIAMA population. Compared to those invited for medical examination at 8 years (n = 1554), children with complete FeNO data at 8 years had a higher level of maternal education and lower prevalence of prenatal smoking. However, differences were small, and with respect to other general characteristics the groups were similar (Table I). Among children with complete FeNO data at the age of 4 years, there was an overrepresentation of the Western study region and an underrepresentation of the Northern and Middle study regions due to technical problems with FeNO measurements. This may explain the somewhat lower proportion of never/infrequent wheeze and late onset wheeze compared to the population invited for medical examination.

**Associations of FeNO values at 4 years and phenotypes of wheeze**

Phenotypes of wheeze were derived from yearly respiratory health assessments from birth up to 8 years. The adjusted geometric mean FeNO was highest in intermediate onset wheeze and persistent wheeze compared to never/infrequent and transient wheeze, but with considerable overlap (Table II).
Table I. General characteristics of study population.

<table>
<thead>
<tr>
<th></th>
<th>Invited for FeNO at 8 yrs (n=1554)</th>
<th>Complete wheezing and FeNO data at 4 yrs (n=588)</th>
<th>Complete wheezing and FeNO data at 8 yrs (n=973)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (% females)</td>
<td>49</td>
<td>48</td>
<td>50</td>
</tr>
<tr>
<td>Study region</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>West</td>
<td>31</td>
<td>50</td>
<td>32</td>
</tr>
<tr>
<td>Middle</td>
<td>37</td>
<td>34</td>
<td>38</td>
</tr>
<tr>
<td>North</td>
<td>32</td>
<td>16</td>
<td>29</td>
</tr>
<tr>
<td>Maternal education level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>22</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>Middle</td>
<td>42</td>
<td>41</td>
<td>42</td>
</tr>
<tr>
<td>High</td>
<td>36</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>Caesarean section</td>
<td>9</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Atopic mother*</td>
<td>64</td>
<td>66</td>
<td>66</td>
</tr>
<tr>
<td>Atopic father*</td>
<td>32</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Exposure to pets in 1st yr</td>
<td>48</td>
<td>47</td>
<td>47</td>
</tr>
<tr>
<td>Older siblings (% present)</td>
<td>48</td>
<td>49</td>
<td>47</td>
</tr>
<tr>
<td>Daycare attendance in 1st yr</td>
<td>24</td>
<td>28</td>
<td>25</td>
</tr>
<tr>
<td>Smoking during pregnancy</td>
<td>16</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Exposure to environmental tobacco smoke†</td>
<td>16</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>Inhaled steroid use†</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Doctors’ diagnosis asthma†</td>
<td>12</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Phenotypes of wheeze†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never/infrequent wheeze</td>
<td>69.9</td>
<td>66.5</td>
<td>68.8</td>
</tr>
<tr>
<td>Transient early wheeze</td>
<td>18.6</td>
<td>21.8</td>
<td>19.7</td>
</tr>
<tr>
<td>Intermediate onset wheeze</td>
<td>3.9</td>
<td>4.4</td>
<td>4.0</td>
</tr>
<tr>
<td>Persistent wheeze</td>
<td>5.1</td>
<td>5.7</td>
<td>5.2</td>
</tr>
<tr>
<td>Late onset wheeze</td>
<td>2.5</td>
<td>1.6</td>
<td>2.2</td>
</tr>
<tr>
<td>Specific IgE inhalant allergen age 8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive for at least 1 of the 6 tested allergens¶</td>
<td>30</td>
<td>30</td>
<td>29</td>
</tr>
</tbody>
</table>

Values are percentages (%). *: Defined as a positive report of hayfever, allergy and/or asthma. †: Reported at the age of 8 years. ‡: Defined using longitudinal latent class analysis as previously described 14, known in 1165/1554 children invited at 8 years. ¶: The following 6 inhalant allergens were tested for: house dust mite (Dermatophagoides pteronyssinus), cats, dogs, grass pollen (Dactylis glomerata), birch, Alternaria alternata. Complete data on FeNO and specific IgE at 8 years in n=792 children.
Table II. Adjusted geometric mean FeNO (ppb) and change in FeNO per phenotype of wheeze at 4 and 8 years.

<table>
<thead>
<tr>
<th>Phenotype of wheeze</th>
<th>FeNO at 4 years (N=588)</th>
<th>FeNO at 8 years (N=973)</th>
<th>Difference in FeNO between 4 and 8 years (N=420)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n*</td>
<td>mean (95% CI)†</td>
<td>n*</td>
</tr>
<tr>
<td>Never/infrequent</td>
<td>391</td>
<td>8.7 (8.3;9.2)</td>
<td>670</td>
</tr>
<tr>
<td>Transient early</td>
<td>128</td>
<td>8.8 (7.9;9.8)</td>
<td>192</td>
</tr>
<tr>
<td>Intermediate onset</td>
<td>26</td>
<td>11.2 (9.5;13.2)+¶</td>
<td>39</td>
</tr>
<tr>
<td>Persistent</td>
<td>33</td>
<td>9.9 (8.5;11.6)+¶</td>
<td>51</td>
</tr>
<tr>
<td>Late onset</td>
<td>10</td>
<td>9.6 (7.6;12.1)</td>
<td>21</td>
</tr>
</tbody>
</table>

Analyses were weighted for the probability that a child belongs to a certain phenotype (individual posterior membership probabilities). *: Frequency (n) of each wheezing phenotype is calculated as the sum of the membership probability of all children for that phenotype. †: Geometric mean FeNO value (95% confidence interval) in ppb per phenotype of wheeze, adjusted for gender, recent symptoms of cold, study region and education of the mother. ‡: p<0.05 for difference in comparison with never/infrequent wheeze. ¶: p<0.05 for difference in comparison with transient wheeze. #: At 8 years FeNO in every phenotype was significantly (p<0.01) different in comparison to all other phenotypes.

Associations of FeNO values at 8 years among different phenotypes of wheeze

The FeNO at 8 years of age differed significantly among all phenotypes. It should be noted that also at 8 years of age, there was considerable overlap in FeNO among all phenotypes. FeNO was highest when wheeze started later in life and persisted longer, in intermediate onset wheeze, persistent wheeze, and late onset wheeze. The adjusted geometric mean FeNO for each wheezing phenotype is given in Table II and the distributions are shown in Figure 3.
Figure 3. Box plots of FeNO at 8 years per phenotype of wheeze. Horizontal lines indicate the median Fractional exhaled Nitric Oxide. Upper/lower limits of the box, outer lines and dots represent the 25th/75th, the 10th/90th, and the 5th/95th percentiles respectively. Data were weighted for the probability that a child belongs to a certain phenotype (individual posterior membership probabilities).

Change in FeNO over time was analysed in the subgroup of children with FeNO measurements both at 4 and 8 years. FeNO in intermediate and late onset wheezers was significantly higher compared to never/infrequent and transient wheezers.

Environmental tobacco smoke and steroid use did not change the association between FeNO and phenotype of wheeze. These variables add up to 6.5% missing data and were not included in the final model to increase power. None of the other potential confounders changed the association by 10%. Steroids were mainly used in intermediate-, late onset- and persistent wheeze, and this might lead to underestimation of the differences between these phenotypes and the reference group. We think that this is not the case because exclusion of children using steroids at 8 years led to similar results of the phenotypes. To investigate whether the association between FeNO and the wheezing phenotypes may be caused solely by the association between FeNO and wheeze at the age that FeNO was measured, we performed sensitivity analyses adjusting for current wheeze at the ages of 4 and 8 years (ISAAC question: reported wheezing symptoms in the past year). This adjustment did not alter the associations between FeNO and phenotypes of wheeze. All analyses were repeated using wheezing phenotypes defined in the subgroup of children with complete data on wheezing at every age from 1 to 8 years, and this produced similar results (data not shown).
FeNO values and phenotypes of wheeze in atopic and non-atopic children

We performed stratified analyses based on atopy of the mother. The associations between FeNO and phenotypes were similar. However, we found a strong and significant interaction with allergic sensitization of the children themselves at the age of 8 years (overall P-value for interaction <0.001). Among children with elevated specific IgE, FeNO levels at 8 years were low in never/infrequent wheeze and transient early wheeze, and significantly elevated in the remaining persistent phenotypes. In children without elevated specific IgE for inhalant allergens, FeNO levels at 8 years were not significantly associated with phenotypes of wheeze. Because the numbers of children with low specific IgE were small for the wheezing phenotypes with persistent symptoms [intermediate onset wheeze (n = 10), persistent wheeze (n = 20), and late onset wheeze (n = 5)], these phenotypes were also combined for this analysis. Table III shows that, among atopic children, all three phenotypes with persistent symptoms had a significantly higher FeNO level than never/infrequent and transient wheeze, while no such association was present in non-atopic children. This interaction is illustrated in Figure 4.

Table III. Adjusted geometric mean FeNO (ppb) at 8 years per phenotype of wheeze stratified for atopy.

<table>
<thead>
<tr>
<th>Phenotype of wheeze</th>
<th>% atopy</th>
<th>n*</th>
<th>mean (95% CI)</th>
<th>n*</th>
<th>mean (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never/infrequent</td>
<td>23.9</td>
<td>410</td>
<td>7.9 (7.5;8.4)</td>
<td>129</td>
<td>13.5 (12.0;15.3)</td>
</tr>
<tr>
<td>Transient early</td>
<td>27.3</td>
<td>117</td>
<td>7.3 (6.5;8.3)</td>
<td>44</td>
<td>11.8 (9.1;15.2)‡</td>
</tr>
<tr>
<td>Intermediate onset</td>
<td>67.7</td>
<td>10</td>
<td>9.0 (7.1;11.4)</td>
<td>21</td>
<td>22.6 (16.7;30.6)‡</td>
</tr>
<tr>
<td>Persistent</td>
<td>51.2</td>
<td>20</td>
<td>8.2 (6.8;9.8)</td>
<td>21</td>
<td>20.9 (15.4;28.3)‡</td>
</tr>
<tr>
<td>Late onset</td>
<td>72.2</td>
<td>5</td>
<td>8.2 (6.0;11.3)</td>
<td>13</td>
<td>29.4 (20.7;41.8)‡</td>
</tr>
<tr>
<td>Combined persistent phenotypes¶</td>
<td>61.1</td>
<td>35</td>
<td>8.4 (7.2;9.8)</td>
<td>55</td>
<td>22.8 (17.8;29.2)‡</td>
</tr>
</tbody>
</table>

We found significant interaction between phenotypes of wheeze and allergic sensitization on FeNO levels (p-interaction < .001). Atopy of the child defined as specific IgE of ≥ 0.70 IU/mL for at least one inhalant allergen at the age of 8 years. Analyses were weighted for the probability that a child belongs to a certain phenotype (individual posterior membership probabilities). *: Frequency (n) of each wheezing phenotype is calculated as the sum of the membership probability of all children for that phenotype. †: Geometric mean FeNO value (95% confidence interval) (ppb) per phenotype of wheeze stratified for atopy at 8 years, adjusted for gender, recent symptoms of cold, study region and education of the mother. ‡: p<0.05 for difference in comparison with never/infrequent wheeze. ¶: Due to the smaller sample size, 3 phenotypes with persistent symptoms (intermediate onset wheeze, persistent wheeze and late onset wheeze) were also combined in this analysis.
Discussion

We examined FeNO at 4 and 8 years in relation to phenotypes of wheeze and atopy. We found that FeNO at 4 years was higher in intermediate onset and persistent wheeze compared to never and transient wheeze. The association between phenotypes of wheeze and FeNO measured at 8 years was much stronger. FeNO at 8 years was significantly higher in persistent phenotypes of wheeze (including intermediate onset, persistent, and late onset wheeze) compared to never and transient wheeze, but only among children with allergic sensitization at 8 years. Smaller but significant differences were observed for FeNO at 8 years among the three persistent phenotypes.

FeNO and wheezing phenotypes

Previous studies have reported increased FeNO in asthmatic children,\(^3,5,20-22\) while others did not confirm this.\(^{23,24}\) A possible explanation for these discrepancies is that ‘asthma’ comprises several
phenotypes that may or may not share the same inflammatory mechanisms. Lumping all these phenotypes together as a single disease entity might hamper our efforts to understand the aetiology and pathophysiology of specific phenotypes. The presence of such differences has been suggested in studies that assessed airway inflammation using bronchoalveolar lavage (BAL). BAL is an invasive procedure and hence not feasible for research purposes in children with mild disease. Only few studies investigated the association between non-invasive surrogate markers of airway inflammation, like FeNO, and wheezing phenotypes in early childhood. Moeller et al. measured higher FeNO levels in wheezing preschool children compared to non-wheezers, in line with our findings. Among wheezing children the authors found higher FeNO levels in children with a positive asthma prediction index, which is suggestive of persistent symptoms. In contrast, we found no differences in FeNO at the age of 4 between transient and late onset wheezers. This may be explained by differences in classification using the asthma predictive index or longitudinal latent class analysis. Brussee et al. found in the PIAMA study only a weak association of FeNO at 4 years with phenotypes of wheeze up to that age, with slightly higher FeNO levels in children who wheezed at the age of 4 years, compared to those who never wheezed. Also in the present study only weak associations between phenotype and FeNO at 4 years were found. This could be explained by an increase in chronic airway inflammation with age, with differences in FeNO becoming detectable only after the age of 4 years. However, the early development of eosinophilic inflammation as an underlying mechanism of persistent wheeze is not well understood. Alternatively, differences in the methods of FeNO measurement at 4 and 8 years might be involved, but we earlier found that these online and offline methods give similar results, so this seems unlikely. At 8 years, FeNO levels were increased in the phenotypes with persistent symptoms compared to never and transient wheezers. Differences in FeNO at 8 years between the three persistent phenotypes (intermediate onset, persistent, and late onset wheeze) were not only smaller, but also significant. These results need to be interpreted with some caution, as significant differences may have resulted from multiple testing. We analysed change in FeNO over time. Despite the small numbers, the rise in FeNO in intermediate onset and late onset wheezers was significantly higher compared to that in never/infrequent and transient wheezers. Possibly the underlying disease process in late onset wheezers leads to a faster increase of eosinophilic inflammation between 4 and 8 years. Elevated FeNO levels were especially pronounced in the phenotypes with onset of wheezing after the age of 2 years.

**FeNO and atopy**

We found a strong association between atopy and FeNO. This is a consistent finding in earlier studies. Some authors have suggested that the association between asthma and FeNO may
be entirely explained by atopy, implying that measuring FeNO is of limited use to assess whether
or not a child has asthma.\textsuperscript{30} The present study showed that FeNO is not simply a marker of atopy,
but that the presence of atopy modifies the association between wheezing phenotypes and
FeNO, which is in line with previous studies.\textsuperscript{6,23} Indeed, FeNO levels differed substantially between
the wheezing phenotypes in atopic children at 8 years. Furthermore, this shows that not only all
wheezing phenotypes occur in atopic and non-atopic children, but that the pathophysiology of
wheeze in these two groups is probably different. As FeNO has been shown to correlate with
eosinophilic airway inflammation, we speculate that a predominant eosinophilic inflammation
might be present selectively in atopic children with persistent phenotypes of wheeze. Other
mechanisms may play a role in the pathophysiology of transient wheeze and of persistent wheeze
in non-atopic children. Possible mechanisms include smaller airway calibre and/or neutrophilic
airway inflammation.\textsuperscript{27}

\textbf{Strengths and limitations}

A strong point of our study is that we assessed wheezing prospectively and that the wheezing
phenotypes were discovered without pre-specified constraints in two large birth cohorts,
using longitudinal latent class analysis.\textsuperscript{13,14} Well-standardized FeNO measurements,\textsuperscript{1} objective
assessment of atopy at 8 years, and the large size of the PIAMA cohort with good followup
allowed us to detect significant differences in FeNO in less common phenotypes, even after
stratification for atopy.

A point of consideration in the interpretation of the data is that some children were using inhaled
steroids while FeNO was measured, and it has been shown that steroids can decrease FeNO.\textsuperscript{2}
However, any such effect seems limited because a sensitivity analysis after exclusion of steroid
users did not change the results. In addition, one should take into account the possibility that the
reported association between FeNO and phenotypes of wheeze solely depends on the relation
between FeNO and current wheeze at the age of 8 years. This seems unlikely, because adjustment
for current wheeze at 8 years did not alter any of the associations between FeNO and phenotypes
of wheeze. The present study used parent-reported wheezing symptoms. This method of
assessing symptoms is widely accepted in epidemiological asthma studies,\textsuperscript{31} but may lead to
misclassification. Because parents were unaware of their child’s FeNO level, any misclassification
of wheezing would be independent of FeNO, resulting in a diluting effect with underestimation of
the true differences in FeNO between the wheezing phenotypes. Furthermore, the small sample
sizes at the age of 4 years should be noted, which may have decreased the power to detect
significant associations between FeNO and the less frequent wheezing phenotypes. This holds
true also for the stratified analyses of FeNO at 8 years, where sample sizes were small, especially
among the non-atopic children. FeNO was not measured in early life and our data can therefore not confirm earlier findings that FeNO might be increased in transient early wheeze at a time when wheeze was still present.32

**Conclusion**
The FeNO measured at 8 years differed among wheezing phenotypes, only in atopic children. Hence, we speculate that the pathophysiology of wheezing phenotypes differs between atopic and non-atopic children. Whether or not eosinophilic inflammation is indeed causally involved in the pathogenesis of specific wheezing phenotypes remains to be shown.
References


25 A plea to abandon asthma as a disease concept. Lancet 2006; 368:705.