

Perinatal risk factors for wheezing phenotypes in the first 8 years of life

D. Caudri¹, O. E. M. Savenije^{2,3,4}, H. A. Smit⁵, D. S. Postma^{4,6}, G. H. Koppelman⁷, A. H. Wijga⁸, M. Kerkhof², U. Gehring⁹, M. O. Hoekstra¹⁰, B. Brunekreef⁹ and J. C. de Jongste¹

¹Department of Pediatrics/Respiratory Medicine, Erasmus University Medical Center – Sophia Children's Hospital, Rotterdam, The Netherlands,

²Department of Epidemiology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands, ³Department of Pediatrics, Beatrix Children's Hospital, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands, ⁴GRIAC Research Institute, Groningen, The Netherlands, ⁵Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands,

⁶Department of Pulmonology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands, ⁷Department of Pediatric Pulmonology and Pediatric Allergology, Beatrix Children's Hospital, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands, ⁸Centre for Prevention and Health Services Research, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands, ⁹Institute for Risk Assessment Sciences, University Medical Centre Utrecht, Utrecht, The Netherlands and ¹⁰Department of General Pediatrics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

¹⁰Department of General Pediatrics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

Clinical & Experimental Allergy

Summary

Background A novel data-driven approach was used to identify wheezing phenotypes in pre-schoolchildren aged 0–8 years, in the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort. Five phenotypes were identified: never/infrequent wheeze, transient early wheeze, intermediate onset wheeze, persistent wheeze and late onset wheeze. It is unknown which perinatal risk factors drive development of these phenotypes.

Objective The objective of the study was to assess associations of perinatal factors with wheezing phenotypes and to identify possible targets for prevention.

Methods In the PIAMA study ($n = 3963$), perinatal factors were collected at 3 months, and wheezing was assessed annually until the age of 8 years. Associations between perinatal risk factors and the five wheezing phenotypes were assessed using weighted multinomial logistic regression models. Odds ratios were adjusted for confounding variables and calculated with 'never/infrequent wheeze' as reference category.

Results Complete data were available for 2728 children. Risk factors for *transient early wheeze* ($n = 455$) were male gender, maternal and paternal allergy, low maternal age, high maternal body mass index, short pregnancy duration, smoking during pregnancy, presence of older siblings and day-care attendance. Risk factors for *persistent wheeze* ($n = 83$) were male gender, maternal and paternal allergy, and not receiving breastfeeding for at least 12 weeks. *Intermediate onset wheeze* ($n = 98$) was associated with a lower birth weight and *late onset wheeze* ($n = 45$) with maternal allergy.

Conclusion and Clinical Relevance We identified different risk factors for specific childhood wheezing phenotypes. Some of these are modifiable, such as maternal age and body mass index, smoking, day-care attendance and breastfeeding, and may be important targets for prevention programmes.

Keywords childhood asthma, latent class analysis, prevention, risk factors, wheezing phenotypes

Submitted 21 August 2012; revised 24 May 2013; accepted 21 June 2013

Correspondence:

Johan C. de Jongste, Erasmus MC/
Sophia Children's Hospital,

Department of Pediatric Respiratory
Medicine, PO Box 2060, 3000 CB
Rotterdam, The Netherlands.

E-mail: j.c.dejongste@erasmusmc.nl

Cite this as: D. Caudri,

O. E. M. Savenije, H. A. Smit, D. S.
Postma, G. H. Koppelman, A. H. Wijga,
M. Kerkhof, U. Gehring, M. O.

Hoekstra, B. Brunekreef and J. C. de
Jongste, *Clinical & Experimental
Allergy*, 2013 (43) 1395–1405.

Introduction

The rapid rise in asthma prevalence at the end of the last century is most likely caused by a change in environmental factors, affecting individuals with a genetic susceptibility for asthma. A crucial window for the

effect of such environmental factors appears to exist in prenatal and early post-natal life [1]. Understanding the mechanisms of asthma development is essential to find targets for primary prevention. Unfortunately, conclusive evidence on the relevant major risk factors is still lacking. A likely explanation for contradictory findings

in previous studies is that 'childhood asthma' does not refer to a single disease entity, but comprises several distinct wheezing phenotypes, each of which may have different risk factors [2].

Much effort has been put in identifying different wheezing phenotypes in childhood. Martinez et al. described longitudinal patterns of 'transient early', 'persistent' and 'late onset' wheeze, based on reported symptoms between the ages of 0–3 and 3–6 years [3]. Henderson et al. more recently used longitudinal latent class analysis (LLCA) to identify different wheezing phenotypes in the Avon Longitudinal Study of Parents And Children (ALSPAC), where wheeze was reported repeatedly from birth until 8 years [4]. The same analysis was applied in the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) study, and the following five wheezing phenotypes were found: never/infrequent wheeze, transient early wheeze, intermediate onset wheeze, persistent wheeze and late onset wheeze. The fact that these phenotypes were identified in two independent birth cohorts with similar characteristics strongly supports their validity [5].

The aim of this study was to investigate modifiable pre- and perinatal risk factors for these five novel wheezing phenotypes. If specific wheezing phenotypes are associated with different risk factors, this would support the hypothesis that they have a differential aetiology and pathophysiology. Understanding of these complex associations may lead to the discovery of targets for primary prevention of specific wheezing illnesses during childhood.

Materials and methods

Study population

The PIAMA study is a prospective birth cohort study in Western, middle and Northern areas of the Netherlands. Recruitment took place in the years 1996 and 1997. The baseline study population consisted of 3963 children. Questionnaires for parental completion, based on the ISAAC core questionnaires, were sent during pregnancy, at 3 months and yearly after birth up to the age of 8 years. Additional Supporting Information on the PIAMA study design may be found in the online version of this article and have been published previously [6]. The study protocol was approved by the medical ethics committees of the participating hospitals.

Phenotypes of wheeze

Wheeze was assessed annually with the question: 'Has your child had wheezing or whistling in the chest in the last 12 months?' at the age of 1–8 years, and answered with yes or no. Therefore, $2^8 = 256$ different

longitudinal patterns of wheeze were possible. To identify children with similar patterns of wheeze over time, LLCA was used. With LLCA wheezing phenotypes were derived in the PIAMA and the ALSPAC birth cohort, and results were previously reported [4, 5]. The derived phenotypes in these independent cohorts were comparable, supporting the validity of the phenotypes [5]. Including only children with complete data on wheezing at every age in the PIAMA cohort, the following five phenotypes of wheeze were identified: never/infrequent wheeze, transient early wheeze, intermediate onset wheeze, persistent wheeze and late onset wheeze.

Perinatal factors

Candidate perinatal risk factors were selected based on previous literature and availability within our data set, and included parental characteristics ($n = 5$), child characteristics ($n = 5$), prenatal exposures ($n = 3$) and post-natal exposures ($n = 6$). Apart from exposure to fine particulate matter ($PM_{2.5}$) and study region, all investigated risk factors were defined using questionnaire data. Maternal age, maternal body mass index (BMI), pregnancy duration, birth weight and exposure to $PM_{2.5}$ were included as continuous variables. Maternal BMI was calculated on the basis of maternal height and weight before pregnancy, as reported by the mother. Study region was considered a possible confounder and included as a variable with four categories. All other items were analysed as dichotomous variables. High educational level was defined as a completed bachelor and/or master study. Breastfeeding was defined positive if the child was breastfed for at least 12 weeks. Individual exposure to $PM_{2.5}$ was assessed as the annual average concentrations in the year 1999–2000 at the birth address, and estimated by land-use regression models. These models were based on four two-week measurements of $PM_{2.5}$ at 40 sites throughout the study area, and details were previously described [7]. A detailed definition of all perinatal factors is given in the Data S1.

Statistical analysis

All analyses were carried out using SAS 9.1 (SAS Institute, Inc., Cary, NC, USA). The association between phenotypes of wheeze and all perinatal factors was investigated using a weighted multinomial logistic regression model (SAS PROC CATMOD). The analyses were weighted for the probability that a child belonged to a certain phenotype (individual posterior membership probability), to minimize bias due to misclassification of the wheezing phenotypes. All factors were inspected adjusting for gender. Secondly, multivariable models were built, adjusted for the following confounding

variables (based on literature and correlations within our data set): gender, parental allergy, pre- and post-natal smoke exposure, breastfeeding, day-care, pregnancy duration, older siblings, birth weight, maternal age, study region, parental level of education. Finally, modification of the associations between all perinatal factors and wheezing phenotypes was investigated for gender of the child, and maternal allergy. These factors were selected because interaction of gender and maternal allergy with several other perinatal factors has been previously reported [8–10].

Results

Study population

Of the 3,963 children included at baseline 3614 were still in follow-up at 8 years and 2810 (71%) had complete data on wheezing at every single year. This was taken as the study population for the univariate analyses. A detailed flowchart is presented in Fig. 1. Table 1 shows general characteristics of the study population ($n = 2810$). This population was comparable to the baseline population ($n = 3963$) with respect to gender, prematurity, birth weight, older siblings and allergy of the father. Children in the current analysis had parents with a higher level of education, more often received breastfeeding and were less likely to have a mother who smoked during pregnancy, compared to the children who were excluded. The percentage of missing data on perinatal factors in the study population was less than 1% for all variables, apart from maternal BMI, which had 6.5% missings. In the multivariable analysis, 13 potentially confounding variables were included. Eighty-two children (2.4%) had a missing value on at least one of these variables; 2728 children were included in the multivariable analyses.

Wheezing phenotypes

The percentages of children for each phenotype are presented in Table 1. Seventy-five percent of all children belonged to the group with never/infrequently wheezed. Of the children who wheeze, the largest group had transient early wheeze (16.7%), late onset wheeze being the least prevalent phenotype (1.7%). Figure 2 depicts the age-specific probability of wheeze for all phenotypes.

Association of perinatal risk factors with wheezing phenotypes

Parental risk factors. A family history of allergy was significantly associated with a higher risk of transient early wheeze, persistent wheeze and late onset wheeze, but not intermediate onset wheeze (Table 2). A higher

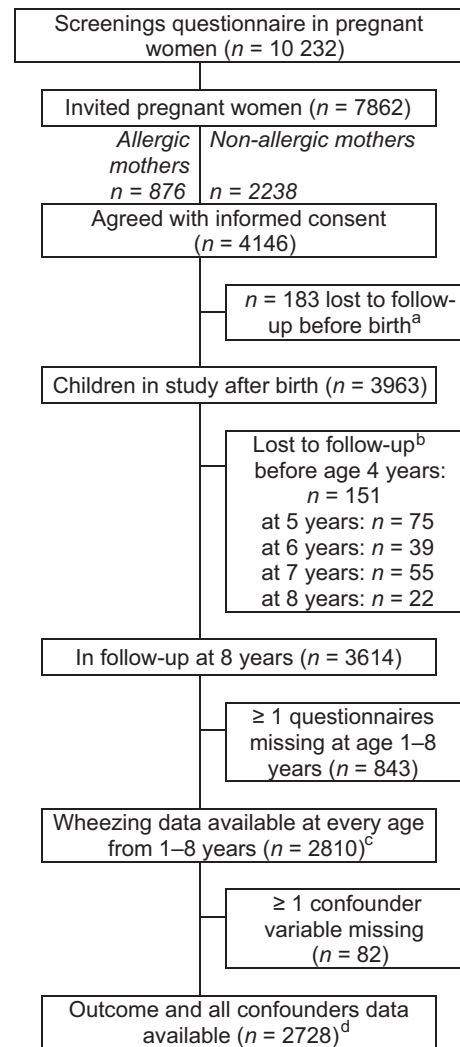


Fig 1. Population flow chart. (a) Lost to follow-up before birth due to various reasons: perinatal death, language barrier, not interested, moved, etc.; (b) lost to follow-up before age 4 due to various reasons (e.g. moved, personal reasons, repeated non-response without known reason); (c) number of subjects used in univariate analyses; (d) number of children included in the multivariable analyses.

maternal BMI was significantly associated with more transient and persistent wheezing.

Child characteristics. Gender, birth weight, pregnancy duration and admittance to a hospital after birth were associated with at least one wheezing phenotype, but caesarean section was not. Boys more often had transient early and persistent wheeze than girls. Children with a lower birth weight were more likely to develop transient early and intermediate onset wheeze. A short pregnancy duration and admittance to a hospital after birth were both associated with more transient early wheeze.

Prenatal exposures. Smoking during pregnancy was the only prenatal exposure variable linked to a wheezing

Table 1. Description of the study population

Variable	n/N	%
General characteristic		
Female gender	1360/2810	48.4
Allergic mother	750/2810	26.7
Allergic father	850/2810	30.3
Maternal education (% high)	1046/2807	37.3
Paternal education (% high)	1166/2792	41.8
Study region		
North	891/2810	31.7
Middle	765/2810	27.2
East	426/2810	16.2
West	728/2810	25.9
Maternal smoking during pregnancy	421/2780	15.1
Premature birth (< 37 weeks)	131/2805	4.7
Low birth weight (< 2500 g)	89/2805	3.1
Breastfeeding ever given	2334/2789	83.7
Older siblings present (≥ 1)	1419/2810	50.5
	Mean/N	IQR
Maternal age (years)	30.7/2804	5.0
Maternal body mass index (kg/m ²)	22.8/2626	3.7
Particulate matter (PM _{2.5}) (ug/m ³)	16.9/2806	2.0
Health outcomes at 8 years		
	n/N	%
Wheezing phenotype*		
Never/infrequent	2109/2810	75.0
Transient early	470/2810	16.7
Intermediate onset	85/2810	3.1
Persistent	99/2810	3.5
Late onset	47/2810	1.7
Allergic sensitization inhaled allergens [†]	389/1434	27.1

*Frequency (n) of each wheezing phenotype is calculated as the sum of the membership probability of all children for that phenotype.

[†]Defined as specific IgE concentration ≥ 0.7 IU/mL for at least one of the following inhaled allergens: house dust mite (*Dermatophagoides pteronyssinus*), cats, dogs, grass pollen (*Dactylis glomerata*), birch, *Alternaria alternata*.

phenotype. It significantly increased the risk for transient early wheeze, and had a non-significant trend for more persistent wheeze with a similar odds ratio (Table 3).

Post-natal exposures. Breastfeeding for at least 12 weeks significantly decreased the risk of persistent wheeze, while older siblings and day-care attendance in the first year increased the risk of transient early wheeze. Environmental tobacco smoke did not significantly increase the risk of any wheezing phenotype. Air pollution as measured by PM_{2.5} was associated with phenotypes with a higher probability of wheezing, especially intermediate onset and persistent, but this did not reach significance.

Multivariable analyses. To exclude confounding by correlated risk factors for wheezing, we performed a

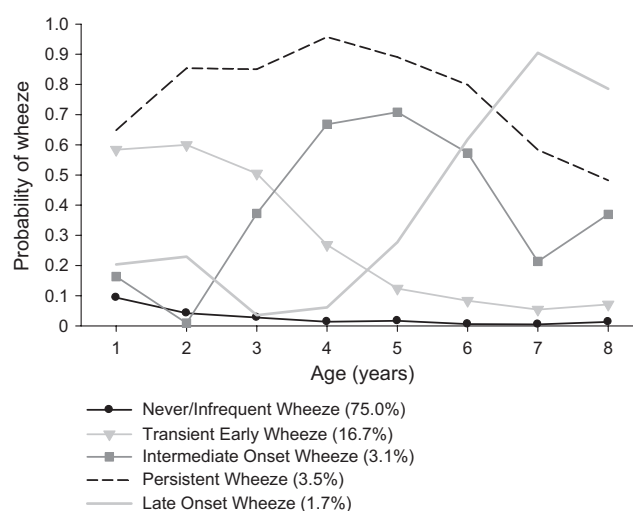


Fig 2. Probability of wheeze at each time-point from birth to age 8 years for each wheezing phenotype in Prevention and Incidence of Asthma and Mite Allergy (N = 2810). The prevalences of the phenotypes are shown next to the phenotypes in the legend. Figure adjusted from Savenije et al. [5] with permission Journal of Allergy and Clinical Immunology.

multivariable analysis. All factors that remained significantly associated with one or more wheezing phenotypes are presented in Fig. 3. In the adjusted analysis, a parental history of allergy and male gender remained the strongest risk factors for wheezing during childhood, and were significantly associated with transient early and persistent wheeze. Two modifiable maternal factors were also associated with wheezing phenotypes. First, children from an older mother were less likely to develop transient early wheeze, but tended to develop more late onset wheeze. Second, a high maternal BMI was significantly associated with transient early wheeze and borderline significantly with persistent wheeze. Low birth weight remained strongly associated with intermediate onset wheeze only, and a short pregnancy with more transient early wheeze. Smoking during pregnancy, older siblings and day-care attendance remained significantly associated with a higher risk of transient early wheeze. Breastfeeding for at least 12 weeks was the only significant protective factor for persistent wheeze.

Effect modification was tested for gender as well as maternal allergy on all perinatal factors listed in Tables 2 and 3, but no significant interaction was found for any of these factors.

Discussion

Wheezing phenotypes during childhood were differentially and sometimes uniquely associated with perinatal risk factors. Allergy of the mother or father, gender,

Table 2. Crude associations of parental and child characteristics at birth with wheezing phenotypes

	Never/infrequent wheeze (<i>n</i> = 2109)	Transient early wheeze (<i>n</i> = 470)	Intermediate onset wheeze (<i>n</i> = 85)	Persistent wheeze (<i>n</i> = 99)	Late onset wheeze (<i>n</i> = 47)
Parental characteristics					
Maternal allergy*					
Frequency, %	24.8	29.5	32.8	40.4	45.0
OR (95 CI)		1.27 (1.02–1.59) [†]	1.49 (0.93–2.36)	2.06 (1.36–3.12) [†]	2.49 (1.39–4.47) [†]
Paternal allergy*					
Frequency, %	28.1	36.2	32.7	40.2	42.3
OR (95 CI)		1.46 (1.18–1.80) [†]	1.25 (0.79–1.99)	1.74 (1.15–2.63) [†]	1.88 (1.05–3.34) [†]
Parental educational level (≥ 1 parent high level)					
Frequency, %	53.5	51.2	51.1	50.3	21.8
OR (95 CI)		0.91 (0.74–1.11)	0.91 (0.59–1.40)	0.87 (0.58–1.31)	0.76 (0.42–1.35)
Age mother [‡]					
Average	30.7	30.4	30.3	30.7	31.1
OR (95 CI)		0.90 (0.79–1.03)	0.83 (0.62–1.11)	1.00 (0.76–1.30)	1.14 (0.78–1.68)
Body mass index mother (kg/m ²)z ^{‡,§}					
Average	22.7	23.1	23.0	23.4	23.0
OR (95 CI)		1.16 (1.03–1.30) [†]	1.10 (0.86–1.42)	1.26 (1.01–1.57) [†]	1.10 (0.79–1.53)
Child characteristics					
Gender (male)					
Frequency, %	50.7	57.8	57.1	66.7	51.7
OR (95 CI)		1.41 (1.15–1.73) [†]	1.37 (0.89–2.12)	2.06 (1.34–3.16) [†]	1.10 (0.62–1.96)
Birth weight (kg)					
Average	3.55	3.48	3.42	3.55	3.48
OR (95 CI)		0.75 (0.63–0.91) [†]	0.63 (0.43–0.93) [†]	0.91 (0.62–1.33)	0.77 (0.45–1.33)
Pregnancy duration (weeks)					
Average	40.0	39.6	39.8	39.7	39.8
OR (95 CI)		0.87 (0.82–0.92) [†]	0.97 (0.85–1.11)	0.91 (0.81–1.02)	0.96 (0.80–1.15)
Caesarean section					
Frequency, %	8.6	8.1	8.9	8.6	9.3
OR (95 CI)		0.92 (0.64–1.33)	1.03 (0.48–2.20)	0.96 (0.47–1.99)	1.08 (0.39–3.00)
Admitted to hospital after birth					
Frequency, %	11.2	15.4	13.0	12.6	13.4
OR (95 CI)		1.41 (1.06–1.70) [†]	1.16 (0.61–2.22)	1.09 (0.59–2.02)	1.22 (0.51–2.90)

Never/infrequent wheeze is reference category, all analyses were weighted for the probability that a child belongs to a certain phenotype (individual posterior membership probabilities). All odds ratios adjusted for gender, not for other possible confounders. OR (95 CI) = Odds ratio with 95% confidence interval.

*Defined positive if any of the following items were reported: asthma, hayfever, house dust allergy, house dust mite allergy or pet allergy.

[†]Associations with a significance level of $P < 0.05$.

[‡]Odds ratio expressed per interquartile range increase.

[§]Based on reported maternal weight and height before pregnancy.

smoking during pregnancy and less breastfeeding were associated with an increased risk factors for all wheezing phenotypes. Pregnancy duration, maternal age, birth weight, day-care and presence of older siblings had differential associations with the various wheezing phenotypes. This may suggest that the underlying pathophysiology is in part similar and in part different between the wheezing phenotypes. Transient early wheeze was significantly associated with several modifiable pre- and post-natal risk factors, namely maternal age, maternal BMI, smoking during pregnancy and day-care attendance in the first year; these factors may be important targets for future prevention programmes.

Risk factors for transient early wheeze

The phenotype transient early wheeze was associated with several perinatal factors, including a family history of allergy, in line with two previous studies on wheezing phenotypes [11, 12]. A possible explanation for the association between parental asthma and transient wheeze in their offspring, could be that these transient wheezers are at increased risk of developing asthma symptoms again at adult age. A long-term follow-up of transient early wheezers confirmed this hypothesis, and suggested it may be explained by tracking of a lower lung function into adulthood [13, 14].

Table 3. Crude associations of perinatal exposures with wheezing phenotypes

	Never/infrequent wheeze (<i>n</i> = 2109)	Transient early wheeze (<i>n</i> = 470)	Intermediate onset wheeze (<i>n</i> = 85)	Persistent wheeze (<i>n</i> = 99)	Late onset wheeze (<i>n</i> = 47)
Prenatal exposures					
Smoking mother during pregnancy*					
Frequency, %	13.9	19.5	18.1	19.1	13.7
OR (95 CI)		1.51 (1.16–1.96) [†]	1.37 (0.78–2.42)	1.48 (0.88–2.48)	0.99 (0.42–2.30)
Antibiotics by mother during pregnancy*					
Frequency, %	8.8	9.7	7.0	10.7	6.7
OR (95 CI)		1.12 (0.80–1.58)	0.79 (0.34–1.84)	1.26 (0.65–2.43)	0.75 (0.23–2.37)
Meconium in amniotic fluid					
Frequency, %	19.7	17.4	17.8	20.5	21.9
OR (95 CI)		0.86 (0.65–1.15)	0.88 (0.47–1.66)	1.07 (0.61–1.85)	1.14 (0.52–2.50)
Early post-natal exposures					
Breastfeeding at least 3 months					
Frequency, %	48.8	43.6	43.3	36.3	43.3
OR (95 CI)		0.82 (0.67–1.01)	0.81 (0.52–1.25)	0.61 (0.40–0.93) [†]	0.80 (0.45–1.44)
Older siblings present (≥ 1)					
Frequency, %	49.3	56.2	45.5	55.7	45.2
OR (95 CI)		1.31 (1.07–1.60) [†]	0.85 (0.55–1.32)	1.28 (0.85–1.92)	0.85 (0.47–1.52)
Day-care attendance in first year					
Frequency, %	24.3	31.5	20.5	19.2	18.3
OR (95 CI)		1.42 (1.14–1.77) [†]	0.80 (0.47–1.37)	0.73 (0.44–1.22)	0.70 (0.33–1.47)
Environmental tobacco smoke exposure [‡]					
Frequency, %	24.5	26.4	27.2	29.4	20.7
OR (95 CI)		1.11 (0.88–1.39)	1.15 (0.70–1.87)	1.29 (0.83–2.01)	0.80 (0.39–1.64)
Pets at 3 months					
Frequency, %	50.0	48.4	50.6	56.2	39.2
OR (95 CI)		0.94 (0.77–1.15)	1.02 (0.66–1.58)	1.28 (0.85–1.92)	0.65 (0.36–1.17)
Particulate matter (PM _{2.5}) at birth address (ug/m ³) [§]					
Average	16.8	17.0	17.1	17.1	17.0
OR (95 CI)		1.15 (0.97–1.35)	1.26 (0.88–1.80)	1.28 (0.92–1.79)	1.17 (0.73–1.88)

Never/ infrequent wheeze is reference category, all analyses were weighted for the probability that a child belongs to a certain phenotype (individual posterior membership probabilities). All odds ratios adjusted for gender, not for other possible confounders. OR (95 CI) = Odds ratio with 95% confidence interval.

*Based on parental reports during second trimester of pregnancy.

[†]Associations with a significance level of $P < 0.05$.

[‡]In the child's house; more than one cigarette per week.

[§]Odds ratio expressed per interquartile range increase in PM_{2.5} concentration.

Multiple modifiable factors were associated with transient wheezing, among which low maternal age and high maternal BMI. Both factors are strongly correlated with socio-economic status, which may explain part of the association. However, the fact that the relations were significant after adjustment for a wide range of potential confounders, indicates that a more direct effect may be present as well. A previous study reported maternal obesity as an independent risk factor for wheezing in early childhood: the authors acknowledged the possibility of residual confounding, but hypothesized that in utero exposure to systemic inflammation in obesity may influence the developing immune system of the child [15]. Another explanation for the association could be that maternal obesity is linked to obesity in their offspring, which may be an independent

risk factor for the wheezing symptoms [16]. Previous studies consistently linked low maternal age with an increased risk of wheezing in childhood, speculating this could be explained by both social and biological factors [17, 18].

Day-care attendance and the presence of older siblings were both associated with transient early wheeze, probably because they increase the risk of respiratory infections [19, 20]. Day-care attendance is a modifiable factor, and given its strong association with transient early wheeze it is a possible candidate for prevention strategies. Importantly, day-care attendance seems to cause a shift in the burden of wheezing illness to an earlier age. The significant increase in transient early wheezing is accompanied by a non-significant trend for less late onset wheeze [11, 12]. Unfortunately, this does

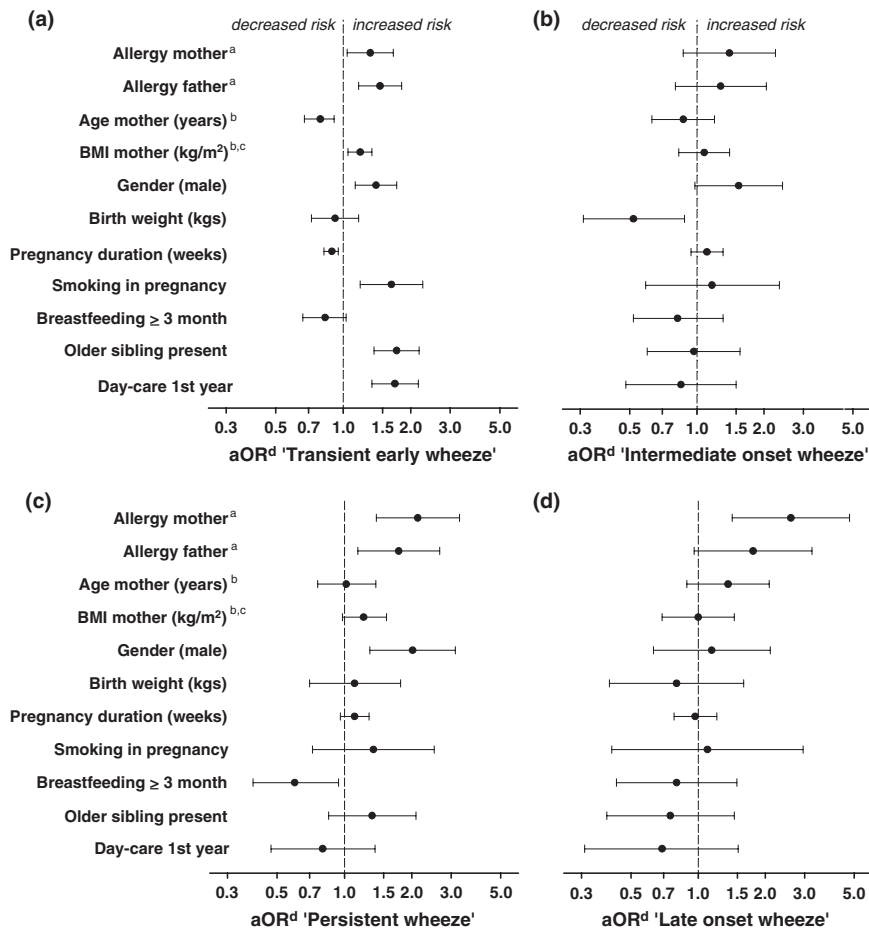


Fig 3. Odds ratios of perinatal risk factors after multivariable adjustment. Never/ infrequent wheeze is reference category, all analyses were weighted for the probability that a child belongs to a certain phenotype (individual posterior membership probabilities). Total $N = 2728$. (a) Defined positive if any of the following items were reported: asthma, hayfever, house dust allergy, house dust mite allergy, or pet allergy, (b) Odds ratios given per interquartile range increase, (c) body mass index, (d) adjusted odds ratio.

not necessarily imply that day-care causes a long-lasting preventive effect on wheezing, as we have previously shown using the PIAMA data [19].

Smoking during pregnancy has been consistently reported as a prenatal risk factor for childhood wheezing [21]. Most studies found a positive association with transient early and/or persistent wheeze [12, 22–24]. Our results are consistent with this in part, and show that in utero smoke exposure was most strongly associated with transient early wheeze. An association with persistent wheeze was not demonstrated in our data. Previous animal studies have demonstrated that tobacco smoke can have a direct negative influence on lung growth and maturation [25]. Apart from this direct effect, prenatal smoking may indirectly cause morbidity mediated by a lower birth weight. We here reported the independent effect of smoking, adjusted for birth weight. Avoidance of prenatal smoking remains a promising preventive intervention as it could lead to a reduction in the prevalence of transient early wheeze

and at the same time reduce the prevalence of low birth weight, which appears to be associated with intermediate onset wheeze.

Risk factors for intermediate onset wheeze

A lower birth weight was the only significant risk factors for intermediate onset wheeze. Birth weight cannot be directly influenced, but several modifiable factors have been reported to influence birth weight, most notably smoking during pregnancy [26]. We cannot easily compare our findings with other studies, because the outcome of intermediate onset wheeze was not investigated previously. Conflicting results on the effect of birth weight on wheezing and asthma in previous studies may well have been caused by differences in outcome definitions, particularly the age of outcome assessment. A large cohort study of 16,000 children in Manitoba, Canada reported more physician-diagnosed preschool asthma in very low birth

weight children before the age of 4 years [27]. A later cohort study of 83 000 children in Alberta, Canada reported 'high birth weight' as risk factor for asthma-related emergency visits in children up to the age of 10 years [28]. We previously reported a negative association in the PIAMA data between birth weight and wheeze until the age of 4 years, which decreased at later ages to unity [29]. The current analysis confirms this time trend and strongly suggests that it is caused by an association of birth weight selectively with the phenotype of intermediate onset wheeze. Intermediate onset wheeze was first described in the ALSPAC study and an independent analysis of the PIAMA data revealed a group of children with a similar pattern of wheeze [4, 5]. The clear differences between risk factors for intermediate onset and other wheezing phenotypes may imply a different underlying pathophysiology. Possibly, children with a lower birth weight are prone to wheezing due to disturbed airway growth in utero and, hence, smaller airways. The fact that the prevalence of wheezing decreases in this group after the age of 5 years fits well with this hypothesis, because children may become less susceptible for wheezing illnesses when their airways increase in size [3]. Why this specific phenotype becomes manifest after a symptom-free interval of several years remains to be elucidated.

Risk factors for persistent wheeze

The most striking risk factor for persistent wheeze was parental allergy, in line with previous literature [3, 11, 12, 22, 23]. Another finding consistent with previous literature is that boys were twice more likely to have persistent wheeze than girls in the studied age range [3, 11, 23]. We found that breastfeeding for at least 3 months significantly reduced the risk of persistent wheezing for at least 8 years, with an odds ratio of 0.6 ($P = 0.03$). Apparently the positive association between breastfeeding and asthma at age 3–8 years that we reported previously was largely explained by our present finding of an association with the phenotype of persistent wheeze. [30]. Odds ratios for all other wheezing phenotypes also showed a non-significant trend towards protection. This implies that breastfeeding reduces the overall prevalence of wheezing, rather than causing a shift in age of onset. Therefore, our data suggest that breastfeeding remains an important target in the prevention of asthma symptoms in the first 8 years of life. Controversy still exists in literature about the effects of breastfeeding on atopic disease, with several observational studies reporting protective effects [31], including one earlier study in the PIAMA cohort [30], while others found increased risks of atopic disease [32]. Differences may to some extent be

explained by differences in the outcome definition as well as the age of assessment. The PROBIT clinical trial did not find a protective effect of breastfeeding on atopic disease [33]. The outcome variables used in this study, 'current' and 'ever wheeze', had a low prevalence in this Eastern European population and this complicates comparisons of these findings to results obtained in settings with higher prevalence of atopic disease. Nevertheless, there remains a possibility that the protective effects of breastfeeding that we found may be explained by residual confounding. Previous studies suggested that there is interaction between breastfeeding, maternal allergy and the risk of asthma. We did not find evidence for this, in line with our previous report [30]. Multiple pathways could explain a lower risk of wheezing in breastfed children. Breast milk may influence the infant's immune system directly by immunomodulatory factors, or indirectly by affecting the infant's mucosal maturation and thereby the intestinal microflora [31, 34].

Risk factors for late onset wheeze

Maternal allergy was the only significant risk factor for late onset wheeze in the multivariate analyses, paternal allergy being of borderline significance. No modifiable risk factors for late onset wheeze were detected. This stresses the importance of genetic constitution in the development of late onset wheeze, but also suggests a lack of perinatal targets for the prevention of it. However, it should be noted that the small sample size may have reduced the power to detect any significant differences. To estimate long-term benefits of any preventive strategies, follow-up is needed to know which of the wheezing phenotypes is most strongly related with asthma symptoms in adolescence and/or adulthood. Late-onset wheezers are often considered as true asthmatics [35], but follow-up of the Tucson cohort has shown that persistent wheezers may actually be more likely to have asthma at adult age than those with late onset wheeze [14].

The presence of pets at 3 months was not associated with any of the wheezing phenotypes. Previous analysis of our data showed a protective effect of pets at 3 months on sensitization, but not on asthma symptoms. [36]. Due to small sample size, we were not able to test the effects of different pets, genetic variants and/or pet avoidance in different phenotypes in the current analysis.

The possible impact of an intervention on a risk factor depends not only on its odds ratio but also on the prevalence of a risk factor in the population. The attributable fraction (AF) takes this into account, and is given in the online supporting information (Table S1). The AF of a risk factor is 'the proportion of children

with a specific wheezing phenotype that could be prevented by eliminating the risk factor from the population', but it is likely an overestimation of the actual effect of an intervention on this risk factor. Therefore, interventions on the risk factors for transient early wheeze given in Table S1 will have only a limited impact, with < 10% reduction in transient early wheeze. Breastfeeding had the highest AF of all modifiable risk factors (25.6%), suggesting that it may indeed be the single most important target for interventions to reduce the prevalence of persistent wheeze.

Strengths and limitations

Strengths of this study are the large sample size with high follow-up rate, and the longitudinal design. Regular follow-up with annual questionnaires that start before birth, eliminated the risk of recall bias. Such bias may have affected the results of the few previous studies investigating risk factors for wheezing phenotypes [12, 22, 24, 32]. We used an unbiased and data-driven approach to prospectively identify several distinct wheezing phenotypes within our cohort. The use of annual recordings of wheeze allowed us to detect new wheezing phenotypes, in a period that wheezing prevalence changes rapidly. Correct classification of wheezing phenotypes remains crucial to understand the complex associations with a wide range of perinatal risk factors.

Some considerations should be made when interpreting our results. We found differential associations with the wheezing phenotypes, suggesting different pathophysiologies. Causality however cannot be proven using observational data. We adjusted our results for many possible confounders, but we cannot exclude that some unknown or residual confounding influenced the results. Furthermore, despite the large size of the PIAMA birth cohort, relatively few children were classified as late onset wheezers, with limited power to detect significant associations with perinatal risk factors. To a lesser extent, this may have also affected the results for intermediate onset and persistent wheeze.

Implications

We found different patterns of risk factors for the newly derived wheezing phenotypes, based on latent class analysis, and this could imply that these phenotypes are indeed different disease entities, with different pathophysiologies. This should be taken into account in the search for prevention strategies. Our data show that most modifiable risk factors may have an influence on transient early wheeze. Prevention of transient early wheeze will have the biggest impact on symptoms in the first years of life. It is the most prevalent wheezing

phenotype and there is evidence that these children are at increased risk for adult asthma as well [14]. We found that prenatal smoking is a promising target for prevention, as it was associated with transient early wheeze and simultaneously causes a lower birth weight, which may lead to more intermediate onset wheeze. Most importantly, we found that the promotion of breastfeeding for at least 3 months has the potential to have a large impact on persistent wheeze, which in turn has a high risk of chronic asthma lasting into adulthood [14].

In conclusion, we examined the association of perinatal risk factors with childhood wheezing phenotypes. We found that specific risk factors were associated with different phenotypes. This suggests that the underlying pathophysiology of phenotypes may be different. We identified the modifiable risk factors maternal age and body mass index, smoking, day-care attendance and breastfeeding. Especially, smoking and breastfeeding are important targets for prevention.

Acknowledgements

We thank the children and parents of the PIAMA study for their participation. Also, we gratefully acknowledge the assistance of Dr. R. Granell, Prof. Dr. A. J. Henderson, Prof. Dr. J. Sterne and Dr. C. W. N. Looman, for providing valuable discussion and assistance in the data analysis.

Author contributions

HAS, DSP, AHW, MK, MOH, BB and JCdJ participated in the study design and/or data collection. All authors participated in the statistical analysis and interpretation of the data. All authors contributed to the writing of the report and have seen and approved the final version of this manuscript.

Conflict of interest

GHK has received a fee (Euro 900, money to institution) for lecturing at a postgraduate course in paediatric asthma from GSK, and has received funding for research from the Dutch Lung Foundation for research carried out in this study. OS received an AGIKO grant from ZonMw (grant nr. 92003555) and reimbursement for attending the ATS congress in 2010 from the Lung Foundation (nr. 3.2.10.085CO). DC, AW, HAS, UG, DSP, MK, MOH, BB and JCJ have no conflicts of interest to declare.

Funding

The PIAMA study is supported by the Netherlands Organization for Health Research and Development; the Netherlands Organization for Scientific Research; the

Netherlands Asthma Fund (AF 3.2.09.081JU); the Netherlands Ministry of Spatial Planning, Housing, and the Environment; and the Netherlands Ministry of Health, Welfare and Sport. The salary of DC was paid by a 'Toptalent' grant from Netherlands Organization for Scientific Research (NWO). None of the funding sources had a role in either the study design, data collection, analyses, interpretation of the data, writing of the

report or decision to submit this manuscript for publication.

Ethical approval

The study protocol was approved by the medical ethics committees of the participating university hospitals.

References

- Bush A. Asthma research: the real action is in children. *Paediatr Respir Rev* 2005; 6:101–10.
- Henderson J, Granell R, Sterne J. The search for new asthma phenotypes. *Arch Dis Child* 2009; 94:333–6.
- Martinez FD, Wright AL, Taussig LM *et al*. Asthma and wheezing in the first six years of life. *N Engl J Med* 1995; 332:133–8.
- Henderson J, Granell R, Heron J *et al*. Associations of wheezing phenotypes in the first 6 years of life with atopy, lung function and airway responsiveness in mid-childhood. *Thorax* 2008; 63:974–80.
- Savenije O, Granell R, Caudri D *et al*. Comparison of childhood wheezing phenotypes in 2 birth cohorts: ALSPAC and PIAMA. *J Allergy Clin Immunol* 2011; 127:1505–12. e14.
- Brunekeerf B, Smit J, de Jongste J *et al*. The prevention and incidence of asthma and mite allergy (PIAMA) birth cohort study: design and first results. *Pediatr Allergy Immunol* 2002; 13 (Suppl 15):55–60.
- Brauer M, Hoek G, van Vliet P *et al*. Estimating long-term average particulate air pollution concentrations: application of traffic indicators and geographic information systems. *Epidemiology* 2003; 14:228–39.
- Celedon JC, Wright RJ, Litonjua AA *et al*. Day care attendance in early life, maternal history of asthma, and asthma at the age of 6 years. *Am J Respir Crit Care Med* 2003; 167:1239–43.
- Mandhane PJ, Greene JM, Sears MR. Interactions between breast-feeding, specific parental atopy, and sex on development of asthma and atopy. *J Allergy Clin Immunol* 2007; 119: 1359–66.
- Wright AL, Sherrill D, Holberg CJ, Halonen M, Martinez FD. Breast-feeding, maternal IgE, and total serum IgE in childhood. *J Allergy Clin Immunol* 1999; 104(3 Pt 1):589–94.
- Midodzi WK, Rowe BH, Majaesic CM, Saunders LD, Senthilselvan A. Predictors for wheezing phenotypes in the first decade of life. *Respirology* 2008; 13:537–45.
- Rusconi F, Galassi C, Corbo GM *et al*. Risk factors for early, persistent, and late-onset wheezing in young children. SIDRIA Collaborative Group. *Am J Respir Crit Care Med* 1999; 1:1617–22.
- Morgan WJ, Stern DA, Sherrill DL *et al*. Outcome of asthma and wheezing in the first 6 years of life: follow-up through adolescence. *Am J Respir Crit Care Med* 2005; 172:1253–8.
- Stern DA, Morgan WJ, Halonen M, Wright AL, Martinez FD. Wheezing and bronchial hyper-responsiveness in early childhood as predictors of newly diagnosed asthma in early adulthood: a longitudinal birth-cohort study. *Lancet* 2008; 372:1058–64.
- Haberg SE, Stigum H, London SJ, Nystad W, Nafstad P. Maternal obesity in pregnancy and respiratory health in early childhood. *Paediatr Perinat Epidemiol* 2009; 23:352–62.
- Scholtens S, Wijga AH, Seidell JC *et al*. Overweight and changes in weight status during childhood in relation to asthma symptoms at 8 years of age. *J Allergy Clin Immunol* 2009; 123:1312–8. e2.
- Martinez FD, Wright AL, Holberg CJ, Morgan WJ, Taussig LM. Maternal age as a risk factor for wheezing lower respiratory illnesses in the first year of life. *Am J Epidemiol* 1992; 136:1258–68.
- Sherriff A, Peters TJ, Henderson J, Strachan D. Risk factor associations with wheezing patterns in children followed longitudinally from birth to 3(1/2) years. *Int J Epidemiol* 2001; 30:1473–84.
- Caudri D, Wijga A, Scholtens S *et al*. Early daycare is associated with an increase in airway symptoms in early childhood but is no protection against asthma or atopy at 8 years. *Am J Respir Crit Care Med* 2009; 180:491–8.
- Marbury MC, Maldonado G, Waller L. Lower respiratory illness, recurrent wheezing, and day care attendance. *Am J Respir Crit Care Med* 1997; 155:156–61.
- DiFranza JR, Aligne CA, Weitzman M. Prenatal and postnatal environmental tobacco smoke exposure and children's health. *Pediatrics* 2004; 113(4 Suppl.):1007–15.
- De Sario M, Di Domenicantonio R, Corbo G *et al*. Characteristics of early transient, persistent, and late onset wheezers at 9 to 11 years of age. *J Asthma* 2006; 43:633–8.
- Kurukulaaratchy RJ, Waterhouse L, Matthews SM, Arshad SH. Are influences during pregnancy associated with wheezing phenotypes during the first decade of life? *Acta Paediatr* 2005; 94:553–8.
- Lewis S, Richards D, Bynner J, Butler N, Britton J. Prospective study of risk factors for early and persistent wheezing in childhood. *Eur Respir J* 1995; 8:349–56.
- Collins MH, Moessinger AC, Kleinerman J *et al*. Fetal lung hypoplasia associated with maternal smoking: a morphometric analysis. *Pediatr Res* 1985; 19:408–12.
- Bailey BA, Byrom AR. Factors predicting birth weight in a low-risk sample: the role of modifiable pregnancy health behaviors. *Matern Child Health J* 2007; 11:173–9.
- Schaubel D, Johansen H, Dutta M, Desmeules M, Becker A, Mao Y. Neonatal characteristics as risk factors for pre-school asthma. *J Asthma* 1996; 33:255–64.
- Sin DD, Spier S, Svenson LW *et al*. The relationship between birth weight and childhood asthma: a population-

- based cohort study. *Arch Pediatr Adolesc Med* 2004; **158**:60–4.
- 29 Caudri D, Wijga A, Gehring U *et al.* Respiratory symptoms in the first 7 years of life and birth weight at term: the PIAMA Birth Cohort. *Am J Respir Crit Care Med* 2007; **175**:1078–85.
 - 30 Scholtens S, Wijga AH, Brunekreef B *et al.* Breast feeding, parental allergy and asthma in children followed for 8 years. The PIAMA birth cohort study. *Thorax* 2009; **64**:604–9.
 - 31 Oddy WH. A review of the effects of breastfeeding on respiratory infections, atopy, and childhood asthma. *J Asthma* 2004; **41**:605–21.
 - 32 Sears MR, Greene JM, Willan AR *et al.* Long-term relation between breastfeeding and development of atopy and asthma in children and young adults: a longitudinal study. *Lancet* 2002; **360**:901–7.
 - 33 Kramer MS, Matush L, Vanilovich I *et al.* Effect of prolonged and exclusive breast feeding on risk of allergy and asthma: cluster randomised trial. *BMJ* 2007; **335**:815.
 - 34 Rubaltelli FF, Biadaioli R, Pecile P, Nicoletti P. Intestinal flora in breast- and bottle-fed infants. *J Perinat Med* 1998; **26**:186–91.
 - 35 Stein RT, Holberg CJ, Morgan WJ *et al.* Peak flow variability, methacholine responsiveness and atopy as markers for detecting different wheezing phenotypes in childhood. *Thorax* 1997; **52**:946–52.
 - 36 Kerkhof M, Wijga AH, Brunekreef B *et al.* Effects of pets on asthma development up to 8 years of age: the PIAMA study. *Allergy* 2009; **64**:1202–8.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Perinatal risk factors for wheezing phenotypes in the first 8 years of life.

Online Supporting Information

Title:

Perinatal risk factors for wheezing phenotypes in the first 8 years of life

Authors:

Daan Caudri

Olga E.M. Savenije

Henriette A. Smit

Dirkje S. Postma

Gerard H. Koppelman

Alet H. Wijga

Marjan Kerkhof

Ulrike Gehring

Maarten O. Hoekstra

Bert Brunekreef

Johan C. de Jongste

Methods

Recruitment of participants

All children participated in the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort study. This multicentre study was conducted in 3 different regions of The Netherlands: north (Groningen and surroundings), central (Bilthoven, Wageningen and surroundings) and southwest (Rotterdam and surroundings). Recruitment took place between March 1996 and May 1997 by means of a validated screening questionnaire,^(S1) distributed by midwives to 10,232 pregnant women visiting one of 52 prenatal clinics. According to the results of this screening the women were divided in an allergic and a non-allergic group. Women with any of the following self-reported symptoms were defined as allergic: asthma, hay fever, house dust allergy, house dust mite allergy or pet allergy. Children of allergic women were defined as 'high-risk'. Based on the screening 7,862 women (2,779 allergic and 5,083 non-allergic) were invited to participate in the study, approximately 50% (n=4,146) agreed and gave informed consent (1,327 allergic and 2,819 non-allergic). The PIAMA cohort includes an intervention part, studying the effect of impermeable mattress covers, and a natural history part. Figure S1 shows the flow diagram for recruitment of the intervention and the natural history arm. In the present study we considered children from both the intervention and the natural history part. As previously published, the intervention had no effect on the incidence of allergy or respiratory symptoms, including the symptom of wheezing.^(S2) After birth, the study population consisted of 3963 children. Eligible for the present study were children with complete data on wheezing at every age from 1-8 years (n=2810). In these

children 5 wheezing phenotypes were previously identified using Longitudinal Latent Class Analysis.(S3)

Perinatal factors

In total 19 possible perinatal risk factors were investigated. The following parental characteristics were included: maternal allergy, paternal allergy, parental educational level, maternal age, and maternal body mass index (BMI). Parents were considered 'allergic' if they reported any of the following items: asthma, hay fever, house dust allergy, house dust mite allergy, pet allergy. Information on education of both parents was combined in one dichotomous variable (low vs. high), and considered 'high' if at least one of the parents completed a bachelor or masters study. Maternal BMI was calculated on the basis of maternal height and weight before pregnancy, and was reported by the mother in the questionnaire sent when the child was 1 year old. The odds ratios of both maternal age and BMI were calculated per interquartile range increase. The child characteristics that were investigated were: gender, birth weight, pregnancy duration, caesarian section, and whether a child was admitted to a hospital directly after birth. The odds ratios for gender were calculated with girls as the reference group. Estimates for birth weight were calculated per kilogram higher weight, and estimates for pregnancy duration per week. Three prenatal exposures were investigated: prenatal smoking, prenatal use of antibiotics (both assessed during the second trimester of the pregnancy), and exposure to meconium in the amniotic fluid which was asked when the child was three months old. The investigated postnatal exposures included breastfeeding, the presence of older siblings, daycare attendance in the first year, environmental tobacco smoke exposure, the presence of pets in the household, and exposure to particulate matter

(PM_{2.5}) at the child's birth address. Breastfeeding (BF) was assessed as the total number of weeks that breastfeeding was given. In a previous PIAMA publication this variable was divided in three categories (no BF, 0-16 wks BF, more than 16 wks BF), but due to the small sample size in some wheezing phenotypes it was dichotomized in this paper: no breastfeeding or less than 12 weeks vs. at least 12 weeks.(S4)

Daycare was defined as at least 4 hours per week in a professional daycare institution, where children were exposed to other children under the age of 12 years. The average group size in professional daycare centers in the Netherlands is 10 children. Environmental tobacco smoke was assessed when the child was three months old, and defined as exposure to tobacco smoke at least once a week in the living room of the child's house.

Statistical analysis

All analyses were carried out using SAS 9.1 (SAS Institute, Inc., Cary, NC). As outcome in this study we used wheezing phenotypes, that were previously derived in the PIAMA study using longitudinal latent class analysis (LLCA). This statistical approach attempts to identify groups of children within the population (latent classes) that have a similar pattern of wheeze over time. This is done by selecting subgroups within which the occurrence of wheeze at a certain time point, is statistically independent of the occurrence of wheeze at another point in time. Rather than appointing every individual child to a single latent class (i.e. phenotype), the model calculates for every child the probability of belonging to each of the identified latent classes. This probability is called the 'individual posterior membership probability'. Since 5 latent classes (wheezing phenotypes) were identified in the PIAMA study, 5 posterior membership probabilities were calculated for every child. The association

between phenotype of wheeze and all perinatal factors was investigated using a weighted multinomial logistic regression model (SAS PROC CATMOD). These multinomial models were weighted for the individual posterior membership probabilities, in order to minimize the risk of bias due to phenotype misclassification.

Attributable Fraction

The aim of our study was to find possible interventions to prevent wheezing illnesses in childhood. In order to assess the maximal possible effect of a prevention program on any modifiable risk factor, it is important to consider the strength of the association with the outcome, but also the prevalence of the risk factor in the population. Both aspects are taken into account in the attributable fraction (AF). The AF indicates the excess number of children with a wheezing phenotype associated with a specific risk factor, and is expressed as a proportion of the total number of children with the wheezing phenotype. We calculated the AF for all modifiable factors that were significantly associated with a wheezing phenotype in the multivariate analysis. The AF of a risk factor was estimated by calculating the expected number of cases using the regression coefficients of the multivariable model, after elimination of that (dichotomous) risk factor from the population.(S5) The AF of continuous variables was estimated by changing the population distribution to a degree that would be clinically plausible. Therefore the following changes were modeled in the continuous variables: 1) maternal age was increased with 5 yrs, but only in mothers < 30 yrs of age; 2) maternal BMI was decreased to a maximum of 25 kg/m² for all mothers; 3) pregnancy duration was increased to a minimum of 39 wks for all children; and 4) birth weight was increased with 200 grams in all children.

Results

Attributable Fraction

The results for the attributable fraction of all risk factors that were significant in the multivariable analysis are given in table S1. Transient early wheeze had the most significant risk factors after multivariable adjustment. Parental allergy and gender were significant risk factors, but as these factors are not modifiable, these are no targets for interventions. The presence of older siblings had the highest AF, but also this factor would in practice not be usable for an intervention. The AF of the other significant risk factors for transient early wheeze was small ($\leq 10\%$). The AF could be interpreted as 'the proportion of children with a specific wheezing phenotype that could be prevented by completely eliminating the risk factor from the population'. However, there are two important assumptions in this interpretation. First, the association of the risk factor with the wheezing phenotype is assumed to be completely causal. Causality cannot be proven using observational data. Results were adjusted for known confounders, but it cannot be excluded that some unknown or residual confounding influenced our results. Secondly, it is unlikely that an intervention in the general population could achieve a complete elimination of any of the investigated risk factors. Therefore the AF is an overestimation of the actual effects of a prevention program on public health. Taking these considerations into account, the proportion of wheezing that may be avoided by interventions on perinatal risk factors is probably limited.

The only significant risk factor for intermediate onset wheeze was birth weight. The most important modifiable factor that can influence birth weight is smoking during pregnancy. In our data smoking during pregnancy led on average to a 200 gram

reduction in birth weight, which is why we calculated the AF by modeling a 200 gram increase in birth weight. The AF of 12.3% is considerable, but should be interpreted with caution, as it may be very difficult to actually influence birth weight on a population level.

Parental allergy and male gender were associated with persistent wheeze, but these factors are not modifiable. Breastfeeding was the only modifiable significant risk factor for persistent wheeze. It had the highest AF (25.6%) of all modifiable factors, which can be explained by the strong odds ratio, in combination with the high proportion of mothers that did not give breastfeeding for at least three months (over 50%). Therefore breastfeeding remains an important target in the prevention of asthma symptoms in the first 8 years of life.

The outcome of late onset wheeze was significantly associated with maternal allergy only. As this is not a modifiable factor, we could not estimate the possible impact of any intervention to reduce late onset wheeze.

References

- S1. Lakwijk, N., R. T. Van Strien, G. Doekes, B. Brunekreef, and J. Gerritsen. 1998. Validation of a screening questionnaire for atopy with serum IgE tests in a population of pregnant Dutch women. *Clin Exp Allergy* 28(4):454-8.
- S2. Brunekreef, B., R. van Strien, A. Pronk, M. Oldenwening, J. C. de Jongste, A. Wijga, M. Kerkhof, and R. C. Aalberse. 2005. La mano de DIOS...was the PIAMA intervention study intervened upon? *Allergy* 60(8):1083-6.
- S3. Savenije, O., R. Granell, D. Caudri, G. Koppelman, J. De Jongste, A. Wijga, H. Smit, D. Postma, J. Henderson, and M. Kerkhof. 2011. Comparison of childhood wheezing phenotypes in 2 birth cohorts: ALSPAC and PIAMA. *J Allergy Clin Immunol* 127(6):1505-12 e14.
- S4. Scholtens, S., A. H. Wijga, B. Brunekreef, M. Kerkhof, M. O. Hoekstra, J. Gerritsen, R. Aalberse, J. C. de Jongste, and H. A. Smit. 2009. Breast feeding, parental allergy and asthma in children followed for 8 years. The PIAMA birth cohort study. *Thorax* 64(7):604-9.
- S5. Nusselder, W. J., K. van der Velden, J. L. van Sonsbeek, M. E. Lenior, and G. A. van den Bos. 1996. The elimination of selected chronic diseases in a population: the compression and expansion of morbidity. *Am J Public Health* 86(2):187-94.

Figure legends

Tables

Table S1. Attributable fraction of risk factors after multivariable adjustment*

Risk factor	Transient Early wheeze (n=455)	Intermediate Onset wheeze (n=83)	Persistent wheeze (n=98)	Late Onset wheeze (n=45)
	----- Attributable fractions -----			
Maternal allergy [‡]	5.9 %	-	20.2 %	28.0%
Paternal allergy [‡]	9.5 %	-	16.0 %	-
Age mother [§]	9.6 %	-	-	-
Maternal BMI (kg/m ²) ^{§¶}	2.2 %	-	-	-
Gender (male)	13.1 %	-	33.0 %	-
Birth weight (per kg)	-	12.3%	-	-
Pregnancy duration (per wk)	3.8 %	-	-	-
Smoking mother pregnancy	5.8 %	-	-	-
No breastfeeding for ≥3 mths	-	-	25.6 %	-
Older sibling present (≥1)	19.5 %	-	-	-
Daycare in first year	10.8 %	-	-	-

Total N=2,728. Never/ infrequent wheeze (n=2047) is reference category.

Frequencies (n) of wheezing phenotypes calculated as the sum of the individual posterior membership probability of all children for that phenotype, all analyses weighted for individual posterior membership probability. *Analyses adjusted for: gender, parental allergy, pre- and postnatal smoke exposure, breastfeeding, daycare, pregnancy duration, older siblings, birth weight, maternal age, study region, parental level of education. ‡: 'Allergy' defined positive if any of the following items were reported: asthma, hay fever, house dust allergy, house dust mite allergy, or pet allergy. §: Odds ratios given per interquartile range increase. ¶: BMI is Body Mass Index.

Figures

Figure S1. The Prevention and Incidence of Asthma and Mite Allergy (PIAMA) recruitment scheme.

