

Received Date : 22-Apr-2015
Revised Date : 10-Sep-2015
Accepted Date : 04-Oct-2015
Article type : Original Article-Asthma and Rhinitis

**STABILITY AND PREDICTIVENESS OF MULTIPLE TRIGGER
AND EPISODIC VIRAL WHEEZE IN PRESCHOOLERS**

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cea.12660
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Key words

Multiple-trigger wheeze, episodic viral wheeze, asthma, preschool children, cohort studies, primary health care

ABSTRACT

Background: In 2008, the European Respiratory Society Task Force proposed the terms multiple-trigger wheeze (MTW) and episodic (viral) wheeze (EVW) for children with wheezing episodes. We determined MTW and EVW prevalence, their 24-month stability and predictiveness for asthma.

Methods: 565 preschoolers (1, 2 and 3-year olds) in primary care with respiratory symptoms were followed until the age of six years when asthma was diagnosed. MTW and EVW status was determined using questionnaire data collected at baseline and after one and two years. We distinguished 3 phenotypes and determined their 24-month stability, also accounting for treatment with inhaled corticosteroids (ICS). Logistic regression was used to analyse the phenotypes' associations with asthma.

Results: 281 children had complete information. MTW and EVW were stable in 10/281 (3.6%) and 24/281 (8.5%), respectively. The odds of developing asthma for children with stable MTW and stable EVW were 14.4 (1.7-119) and 3.6 (1.2-11.3) times greater than for children free of wheeze (for at least one year). ICS was associated with increased stability of MTW and EVW.

Conclusions: Stable multiple-trigger and stable episodic viral wheeze are relatively uncommon. However, one to three year olds with stable MTW are at much increased risk of asthma.

INTRODUCTION

In 2008, the European Respiratory Society Task Force proposed the terms multiple-trigger wheeze (MTW) and episodic (viral) wheeze (EVW) for children with wheezing episodes to be used to guide treatment. MTW was defined as wheeze that shows discrete exacerbations, but also symptoms between episodes. EVW was defined as wheeze during discrete episodes, often in association with clinical evidence of a viral cold, with absence of wheeze between episodes. Although the Task Force introduced MTW and EVW to be used to guide treatment, the Task Force also stated that their recommendation was based on limited evidence and that children may change between phenotypes over time ¹.

Ideally, the evidence needed to underpin claims to guide treatment consists of systematic reviews and meta-analyses of a large number of randomised trials consistently showing modification of treatment effect by these phenotypes. Until now, studies designed to underpin these claims are lacking. However, over the last years, EVW ²⁻⁷ and MTW ⁸⁻¹¹ have been studied in more detail in terms of lung function levels, skin prick test results, and airway inflammation. Nevertheless, few clinical studies assessed how common these phenotypes are ¹²⁻¹⁵ and, in particular, the extent to which these phenotypes are stable over time ¹⁴. In addition the predictiveness for asthma of these phenotypes has not been studied extensively.

To obtain more evidence to underline the claims stated by the Task Force, we investigated the stability and prevalence of MTW and EVW in a prospective cohort study among preschool children in primary care with a history of wheeze, dyspnoea and/or cough. Moreover, we determined whether the stability of MTW and EVW was associated with the use of ICS. In addition, we determined to which extent these phenotypes are associated with the risk of asthma at the age of six years.

METHODS

Study population and study design

All children in the study were participants of the ARCADE study, a cohort study with 1 to 5-year old children at risk of developing asthma. A detailed description of the study has been published elsewhere ¹⁶. Briefly, between October 2004 and July 2006, we recruited 1 to 5-year old children from 14 general practices in three areas in The

Netherlands. Children were eligible if they had visited a general practitioner with recurrent coughing (≥ 2 visits), wheezing (≥ 1 visit) or shortness of breath (≥ 1 visit) in the year before inclusion. 771 children were included and were followed until the age of six years. There were 432 (56%) boys; 267 (35%), 220 (29%), 127 (17%), 121 (16%), and 36 (5%) were aged 1 through 5, respectively; 518 (67%) had had one symptom (cough: 420; wheeze: 57; dyspnoea: 41), 195 (25%) had had two symptoms (wheeze and cough: 121; wheeze and dyspnoea: 19; cough and dyspnoea: 55); 58 (8%) had had all three symptoms¹⁶.

Demographic data and data with respect to the child's clinical history were collected by questionnaires. Parents were requested to fill in questionnaires yearly until the child reached the age of six years to obtain date of birth, sex, ethnicity, breastfeeding, family history of allergy, asthma and eczema, education, parents' smoking behaviour, housing conditions, and presence of pets. Data on asthma-related symptoms were collected yearly using the Core Questionnaire of the International Study of Asthma and Allergy in Children (ISAAC)¹⁷. Longitudinal data regarding inhaled corticosteroid (ICS) prescriptions – having Anatomical Therapeutic Chemical (ATC) Classification system code R03 – were extracted from the medical records of the child's general practitioner. Use of ICS was defined as having had at least one prescription of ICS during a 12-month follow-up period for the analyses at one year, and as having had at least one prescription of ICS during a 24-month follow-up period for the analyses at two years. In addition, at age six years, presence of asthma was formally assessed (see below). We analysed the data of all 1, 2 and 3-year old children who completed the three questionnaires (baseline, 12, and 24 months). Moreover, these children were followed-up until six years of age.

Phenotype definition

Based on the information collected yearly by questionnaire, multiple-trigger wheeze (MTW) was defined as answering affirmatively to two questions: (i) "Has your child wheezed while having a cold in the previous 12 months?" and (ii) "Has your child wheezed while not having a cold in the previous 12 months?". Episodic viral wheeze (EVW) was defined as an affirmative answer to the first question and a negative answer to the second. Children with lower respiratory tract symptoms but no wheezing were defined as having non-wheezing airway symptoms (NWA). These definitions concur with how these phenotypes are identified in daily practice.

Classification of phenotypes during 24 months of follow-up

For each child, presence of MTW, EVW and NWS was determined at baseline, 12 and 24 months. Then we distinguished the following: (i) stable MTW (MTW at all three time points), (ii) stable EVW (EVW at all three time points), (iii) stable mixed wheezing (either MTW or EVW at all three time points, but not stable MTW or stable EVW), (iv) non-stable wheezing (NWS on ≥ 1 of the three time points, but not stable non-wheezing), and (v) stable non-wheezing (NWS at all three time points). Appendix I shows the details.

Asthma at age six years

For the diagnosis of asthma to be made we required (i) airway hyperresponsiveness to methacholine and (ii) current symptoms and/or use of asthma medication during the previous 12 months. Airway hyperresponsiveness was defined as $PC_{20} FEV_1 \leq 8.0$ mg/ml, or $> 10\%$ increase in FEV_1 after administration of rapid acting beta-2-agonists^{18;19}. Detailed descriptions of both measurement techniques were previously published¹⁶.

Statistical analysis

Proportions of children with stable EVW and MTW were determined and their corresponding 95% confidence intervals (CIs). Stability of EVW and MTW was expressed using the numbers and percentages of children who (had not) moved to another phenotype after 12 and 24 months. Transition (probability) was also expressed graphically. We explored the hypothesis that phenotypic stability increases with age at recruitment using two logistic regression analyses in which stability at one and 2 years, respectively were regressed against age (children aged 1 year at recruitment serving as the reference category). We employed logistic regression to investigate the association of different phenotypes with the presence of asthma at the age of six years. As our goal was to determine the risk of asthma for several phenotypes that were stable over time, we used *non-stable wheezing* as the reference category. Logistic regression yielded odds ratios (ORs) and their corresponding 95% CIs. All calculations were performed using Stata version 10.1 (Stata corp, College Station TX, USA).

We determined whether the phenotypic stability was associated with the use of ICS by splitting up the group of children into those who had been prescribed ICS and those who had not been prescribed ICS in the one (stability over one year) or two years (stability over 2 years) prior the time of analysis and comparing phenotypic stability patterns. Finally, differences in proportions of children in the three age groups, sex, having any siblings, parental

smoking, breastfeeding, family history of asthma or allergies, current exposure to pets and IgE antibodies against cats, dogs and house dust mites between the three phenotypic categories (MTW, EVW and NWAS) were tested using the Chi square test.

RESULTS

Of the 771 children in the cohort, 565 (73.3%) were aged 1, 2 or 3 years old and had complete information at baseline (figure 1). Of these 565 preschool children, 95 (17%) had MTW at baseline, 234 (41%) EVW, and 236 (42%) NWAS. Table 1 shows that age, sex, parental smoking in the home, having siblings, family history of parental allergy, and exposure to pets did not vary significantly by phenotype at baseline. However, a family history of asthma was most prevalent among children with MTW ($P=0.001$). Among children with NWAS, breastfeeding was most common ($P=0.010$). Overall, 281 children had complete phenotype information during the 24-month follow-up period (table 2 and figure 2). For 206 children, complete information including the asthma diagnosis was available.

Stability and prevalence of MTW and EVW

At baseline, of the 281 children with complete follow-up, 49 (17%) had MTW, 126 (45%) EVW and 106 (38%) NWAS. During the follow-up, all children experienced airway symptoms and could be classified as MTW, EVW or NWAS. Of the 49 children with MTW at baseline, 22 (45%) and 11 (22%) had MTW at 12 and 24 months, respectively. Of the 126 children with EVW at baseline, 67 (53%) and 24 (19%) had EVW at 24 months, respectively. Prevalences for stable MTW and stable EVW were 3.6% (95% CI from 1.9 to 6.4) and 8.5% (95% CI from 5.8 to 12.4), respectively. We found weak to moderate evidence that phenotypic stability increased with age at recruitment. In particular, for stability in the first year of follow-up, we found that, compared to children who were 1 year old at recruitment, the odds of changing phenotype in 2-year olds was 0.722 (95%CI from 0.450 to 1.160), and 0.518 (0.294 to 0.913) in 3-year olds, respectively. However, this age trend was not statistically significant, $p=0.275$. For stability at 24 months, the odds ratios were 0.576 (0.353 to 0.939) and 0.810 (0.448 to 1.464). The age trend was not statistically significantly, $p=0.268$.

Use of ICS and phenotype misclassification

Sensitivity analyses were performed to assess the robustness of phenotype classification. We hypothesised that ICS suppressed wheezing which might cause children with MTW or EVW at baseline to be classified as NWAS during follow-up. Of all children with MTW at baseline (49/281), 10/49 (20.4%) could be categorised as stable MTW. Subsequently, 21/49 (42.9%) of the children with MTW at baseline were treated with ICS during a 24-month follow-up period (Appendix II; figure 1). Additionally, of all children with MTW at baseline, 7/21 (33.3%) of the children with a prescription of ICS were categorised with stable MTW during 24-month follow-up period compared to 3/28 (10.7%) of the children with no ICS prescription (difference 22.6% (95%CI -0.6 to 45.8)).

Figure 2 shows that 45% (126/281) of the children had EVW at baseline of which 24/126 (19%) could be categorised as stable EVW (see figure 2). Additionally, Appendix II, figure 1 shows that 42/126 (33.3%) of the children with EVW at baseline received a prescription of ICS during a 24-months follow-up period. Of the children with EVW at baseline, 13/43 (31%) of the children with a prescription of ICS were categorised as stable EVW during the 24-month follow-up against 11/81 (13.1%) of the children that were not treated with ICS during this period (difference 17.9% (95%CI 2.1 to 22.6)). For the children who did not wheeze during the 24-month follow-up at all (stable non-wheezers), we found a difference of -37.4 (95%CI -63.0 to -11.7).

Additionally, we determined whether prescription of ICS was associated with stability of MTW or EVW. Prescription of ICS was associated with MTW stability (odds ratio 4.2; 95%CI 1.0 to 17.2) and EVW stability (odds ratio 3.0; 95%CI 1.2 to 7.3).

Wheezing phenotypes and probability of asthma at age six

For 206 children a formal asthma status at 6 was available. Table 3 shows the risk of asthma at age six for the different phenotypes. In total, 83 (40.3%) children were diagnosed with asthma. Compared to non-stable wheezers, the odds of asthma were increased by a factor 14.4 (95%CI 1.7 to 119.1) and 3.6 (95%CI 1.2 to 11.3) for stable MTW and stable EVW, respectively. For stable mixed wheezing this number was 5.4 (95%CI 1.6 to 17.8).

DISCUSSION

Main findings

The ERS Task Force introduced the phenotypes multiple-trigger wheeze (MTW) and episodic viral wheeze (EVW) to be used in clinical practice. Based on these phenotypes the ERS Task Force formulated several treatment recommendations for clinicians. In this study with preschool children who visited their general practitioner for (recurrent) respiratory symptoms and were followed for 24 months we found that MTW as well as EVW tended to be unstable over time. Eighty percent of the children who had MTW at baseline did not have MTW during the two following years. In children with EVW at baseline this percentage was 81%. We also demonstrated that 75% of the non-wheezing children at baseline remained non-wheezing for two years. We found evidence supporting the hypotheses that, compared to non-stable wheezers, children with stable MTW, but also children with stable EVW or with mixed stable wheezing, are at increased risk of having asthma at age six. Of all children starting as MTW, 49% ended up as NWAS after 2 years, compared to 68% of children starting as EVW (Figure 2, upper two panels). This seems to confirm our clinical experience that EVW children may stop wheezing altogether and generally have no strong tendency to become asthmatic, whereas (stable) MTW children are more likely to keep wheezing and to develop asthma. The regression analysis also confirms that stable EVW children are 4 times less likely to become asthmatic than stable MTW children (odds ratios of 3.6 and 14.4, respectively).

Strength and limitations

Our study has a number of strengths. First, prospective cohort studies of preschool children in a general practice setting are rare. In this study, we assessed the prevalence and two-year (in)stability of MTW and EVW in a high-risk population of preschool children attending general practice. We determined the risk of asthma at the age of 6 years for these children using a uniform asthma definition. Second, presence of MTW, EVW or no symptoms of wheeze may be influenced by the use of ICS since ICS may suppress airway symptoms and thus cause misclassification. Therefore, we also determined whether the phenotypic stability depended on the use of ICS and found that the latter was not associated with the instability of the phenotypes. On the contrary, the use of ICS was associated with increased stability of both MTW and EVW.

We see the following limitations to our study. First, the phenotypes MTW and EVW were operationalised based on two questions from a yearly questionnaire. Although the ERS Task Force proposed the phenotypes MTW and EVW, they did not specify the measurement procedures. We therefore used an operationalisation which is also used by others and mirrors the way these phenotypes are sometimes determined by general practitioners^{14,20}. Additionally, by using annually questionnaires we were able to gather information regarding airway symptoms of all preschool children in the cohort, also of those who did not visit their GP for their symptoms. Second, preschool children were classified as MTW and EVW (or NWAS) based on a retrospective questionnaire to the child's parents. This is prone to several sources of potential misclassification. For example, since parents were asked to recall their child's symptoms yearly, single episodes of MTW or EVW could have been missed²¹. In addition, compared to the definitions of wheeze as used by trained medical personnel, parents' interpretation of breathing sounds may be erroneous with sensitivities and specificities below 1. These sources of misclassification may have affected the (prevalence) estimates of (the stability of) the phenotypes. Finally, not all questionnaires were returned by the parents and we were therefore not able to classify all children. However, we think that this drop-out is unlikely to have biased our results since a comparison at baseline between children with all yearly questionnaires and those with at least one questionnaire missing showed minimal differences in characteristics at baseline. To explore differential attrition in more detail, we performed a regression analysis to gain more insight in which variables predicted attrition. Children who dropped out were coded as 1, those remaining in the study as 0. We ran a multivariable logistic regression model with the 9 variables from table 1 as predictors of attrition. We found that there were no predictors that predicted attrition statistically significantly, with p-values ranging from 0.120 (odds ratio=0.685; parental allergies were non-significantly associated with staying in the study) and 0.867 (odds ratio = 0.950; breastfeeding was weakly associated with staying in the study).

Results in relation to other studies

The ERS Task Force introduced MTW and EVW because phenotypes used in epidemiological studies such as transient and persistent wheeze can only be applied retrospectively and are therefore of little use to clinicians¹. Although MTW and EVW can be determined directly, little attention was given to whether these phenotypes are stable over time, which seems an important feature for a patient characteristic assumed to be a modifier of drug effects. Shultz et al. assessed to which extent the phenotypes EVW and MTW are stable over time and showed that

54.1% of the preschool children classified as EVW or MTW, at baseline, had changed phenotype after a 12-month follow-up period ¹⁴. This corresponds with our results indicating that 54.8% and 46.8% of the children with MTW and EVW, respectively, changed phenotype after 12 months. We also showed that after 24 months only 20% of the children with MTW or EVW had been stable over time. The ERS Task Force introduced the phenotypes MTW and EVW to be used in clinical practice and formulated several treatment recommendations for children with MTW and EVW. Since the majority of the children with MTW and EVW changed phenotype after a 12 month period and 80% of the children were not stable after 24 month follow-up, differentiating between symptoms of wheezing in MTW and EVW as proposed by the Task Force stated appears to have limited applicability for decision making in clinical care.

In principle, the use of inhaled corticosteroids may lead, through suppression of wheezing, to inadvertent misclassification of wheezing phenotypes. Although other studies have shown that the influence of ICS on phenotypic misclassification seems to be small ^{1;22} and does not have an effect on the likelihood of developing asthma ^{23;24}, we found that the use of ICS was associated with increased phenotypic stability of both MTW and EVW, and decreased stability of non-wheeze, probably through confounding by indication. In our study, we did not differentiate between children with long-term persistent use of ICS and short intermittent use. Although long-term use of ICS may be expected to influence phenotype expression, we did not differentiate between the intensity of treatment due to the small number of children who received ICS.

So far, several studies have investigated EVW and MTW in preschool children. However, the majority of these studies were performed in general population birth cohorts and studied various strategies for treatment ²⁻¹¹. Studies that investigated the probability of asthma (later in life) of children from clinical care for different wheeze phenotypes are rare. In our cohort, we showed that preschool children in primary care with episodes of MTW and EVW (i.e. stable MTW, stable EVW and stable mixed wheezing) during a 24-month follow-up had an increased risk of asthma at age six. Since, the number of children with stable MTW, stable EVW and stable mixed wheezing was small, 9 (4.4%), 15 (7.3%) and 16 (7.8%), respectively, we also determined the risk of asthma for two additional and larger categories 'ever MTW' and 'never MTW' indicating that children experiencing symptoms of MTW during part of the 24 months follow-up continue experiencing symptoms later in life ⁸⁻¹¹. Our analyses showed that children with 'ever MTW' had an increased risk for asthma at age six compared to children that never wheezed during the 24-month follow-up period. However, differences between children with 'ever MTW' and 'never MTW' were not significant (results presented in Appendix III). Our results concur with those of Kappelle et al. who showed, in a clinical cohort

of preschool children, that the majority of the children (<4 years) who were referred by their general practitioner to a paediatrician because of episodic viral wheeze (defined as 'severe EVW') had asthma at the age of 5 - 10 years¹³.

Currently, the use of asthma prediction models including wheeze but also patient characteristics like age, allergy status and family history of asthma or allergy appear to be more accurate in predicting the development of asthma later in life.

More evidence about the differences between MTW and EVW versus longitudinally derived phenotypes (like transient and persistent wheezing) was provided by Savenije et al. In two large birth cohorts (Avon Longitudinal Study of Parents And Children (ALSPAC) and Prevention and Incidence of Asthma and Mite Allergy (PIAMA study)), Savenije et al. showed that EVW and MTW do not match with the longitudinally derived wheeze phenotypes of transient early wheeze and persistent wheeze, respectively¹⁵. This result seems important since several studies use these terms (EVW and transient early wheeze versus MTW and persistent wheeze) interchangeably¹⁰. Careful attention to the population under study is critical in the interpretation of the literature.

Conclusions

In conclusion, MTW and EVW in preschool children tend not to be stable over time. Eighty percent of these children switched phenotype over a 24-month period of time. This phenotypic instability of MTW and EVW limits their use for treatment selection purposes by clinicians. Nevertheless, one to three year olds with stable MTW are at much increased risk of asthma.

Acknowledgements

The authors would like to thank the general practitioners from HAG-net-AMC, Zorggroep Almere and Prinsenhof, Leemhuis and Buitenhuis for their time and effort in this study. We also thank all children and their parents for participating in the study. We also thank Pauline van Steenwijk, Machteld IJff, Alice Karsten, and Albertien Buijs for their logistical assistance.

Competing interest

The author(s) declare that they have no competing interests.

Ethics approval

The study was approved by the Central Committee on Research Involving Human Subjects (CCMO/P04.0098C).

Written informed consent was obtained from the parents prior to all measurements.

Funding

This study was financially supported by the Netherlands Lung Foundation (3.4.02.20 and 3.4.06.078) and by the Stichting Astma Bestrijding.

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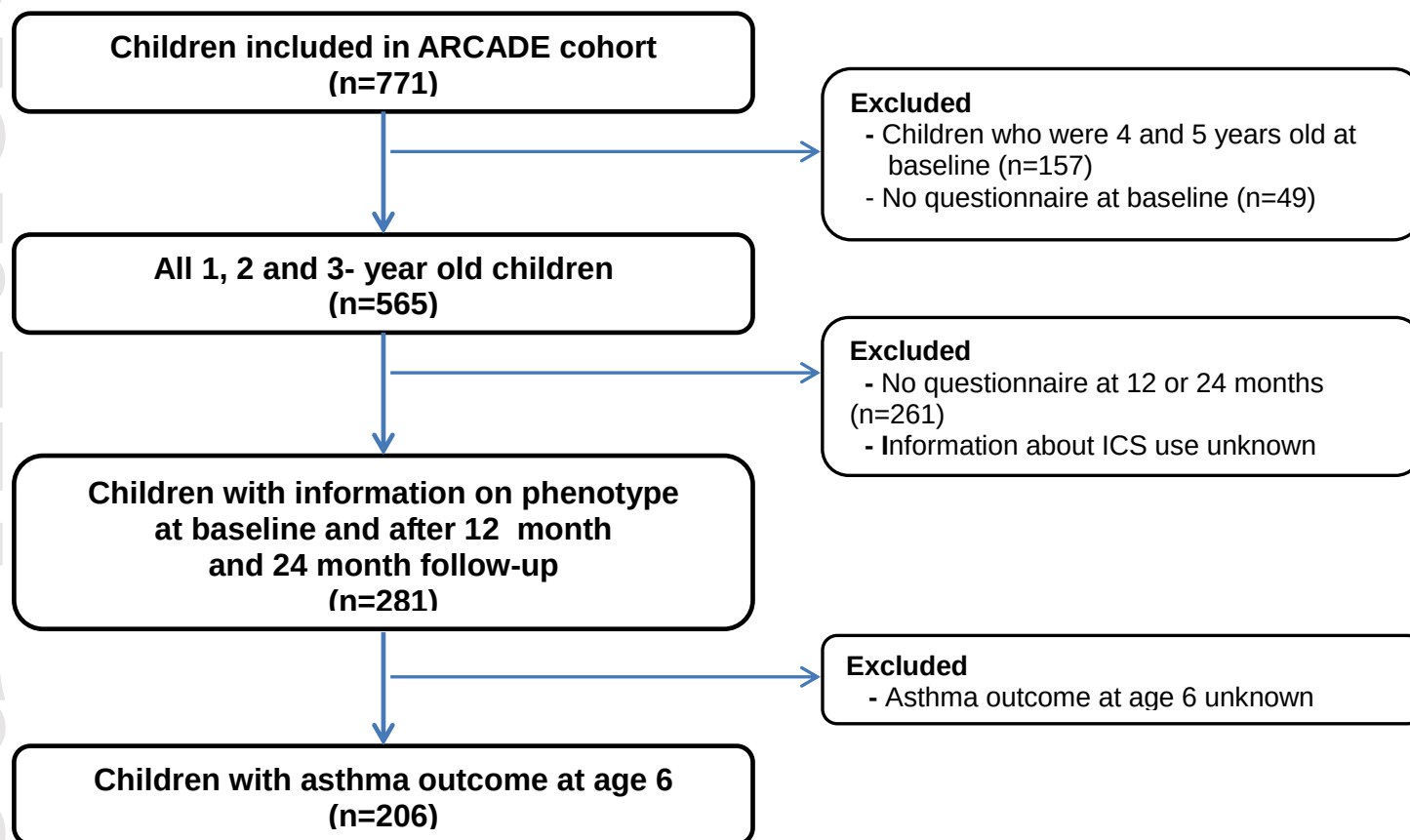
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FIGURES AND TABLES

Figure 1 Flowchart of children in the study



Abbreviations: ICS, inhaled corticosteroids

	PHENOTYPE AT BASELINE (n=565)		
	MTW (n=95; 16.8%)	EVW (n=234; 41.4%)	NWAS (n=236; 41.8%)
Age (years)			
1	43 (45.3)	108 (46.2)	98 (41.5)
2	34 (35.8)	78 (33.3)	91 (38.6)
3	18 (18.9)	48 (20.5)	47 (19.9)
Sex, male	57 (60.0)	132 (56.4)	122 (51.7)
Parental smoking ^a	40 (44.0)	101 (46.1)	86 (38.1)
Siblings ^b	57 (60.0)	146 (62.7)	146 (61.9)
Breastfeeding ^c	63 (66.3)	182 (77.8)	192 (81.7) *
Exposure to pets ^d	34 (35.8)	83 (35.5)	84 (35.7)
Family history of asthma ^e	41 (43.2)	88 (37.6)	58 (24.6) *
Family history of parental allergy ^f	56 (59.2)	118 (50.4)	108 (45.8)
Specific IgE against cats, dogs, and/or house dust ^g	6 (12.2)	23 (17.4)	21 (18.3)

Table 1: Characteristics of the study population at baseline

Abbreviations: MTW, Multiple-trigger wheeze; EVW, Episodic viral wheeze; NWAS, Non-wheezy airway symptoms; IgE, Immunoglobulin E

Numbers are n (percentages in parentheses)

Variables defined as:

^a Does at least one of the parents smoke in the parental house? In total, for 29 children information on *parental smoking* was missing (4 (4.2%), 15 (6.4%),

10 (4.2%) missing values for MTW, EVW, and NWAS, respectively).

^b Does your child have younger or older siblings? One missing value for EVW

^c Has your child been breastfed? One missing value for NWAS

^d Are there any pets in the house? One missing value for NWAS

^e Does at least one of the parents or siblings have asthma?

^f Does at least one of the parents have allergies for pets, pollen and/or house dust mites?

^g Specific IgE positivity to house dust mite, cat and/or dog dander was defined as > 0.35 kU/l. In total, for 269 children information on specific IgE was missing

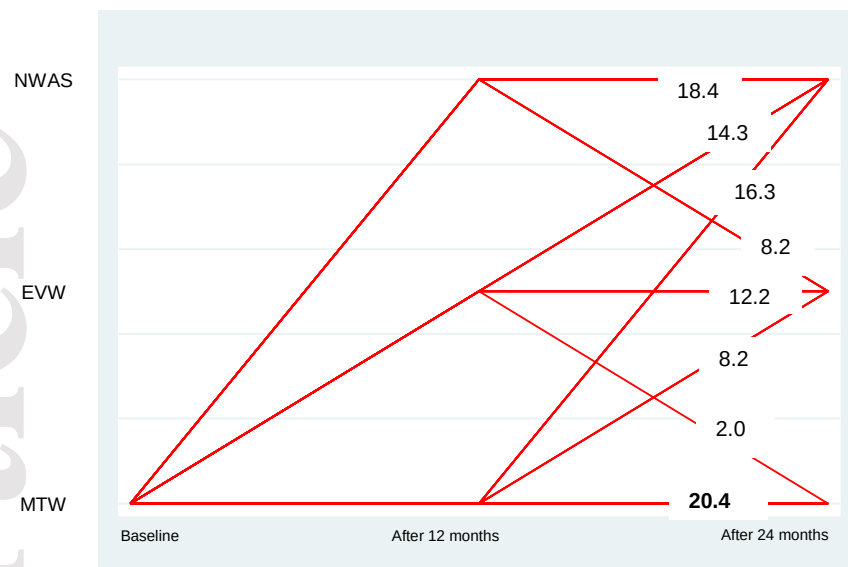
(46 (48.8%), 102 (43.6%), 121 (51.3%) missing values for MTW, EVW and NWAS, respectively).

* Significant: P=0.010 (breastfeeding), and P=0.001 (family history of asthma)

PHENOTYPE AT BASELINE	PHENOTYPE AT 12 AND 24 MONTH FOLLOW-UP (n=281)					
	At 12 month follow-up (baseline versus 12 months)			At 24 month follow-up (baseline versus 24 months)		
	MTW	EVW	NWAS	MTW	EVW	NWAS
MTW (n=49)	22 (44.9)	14 (28.6)	13 (26.5)	11 (22.4)	14 (28.6)	24 (49.0)
EVW (n=126)	9 (7.1)	67 (53.2)	50 (39.7)	7 (5.6)	33 (26.2)	86 (68.3)
NWAS (n=106)	6 (5.7)	13 (12.3)	87 (82.1)	3 (2.8)	14 (13.2)	89 (84.0)
Table 2: Phenotype classification at baseline, 12 and 24 month follow-up (n=281)						

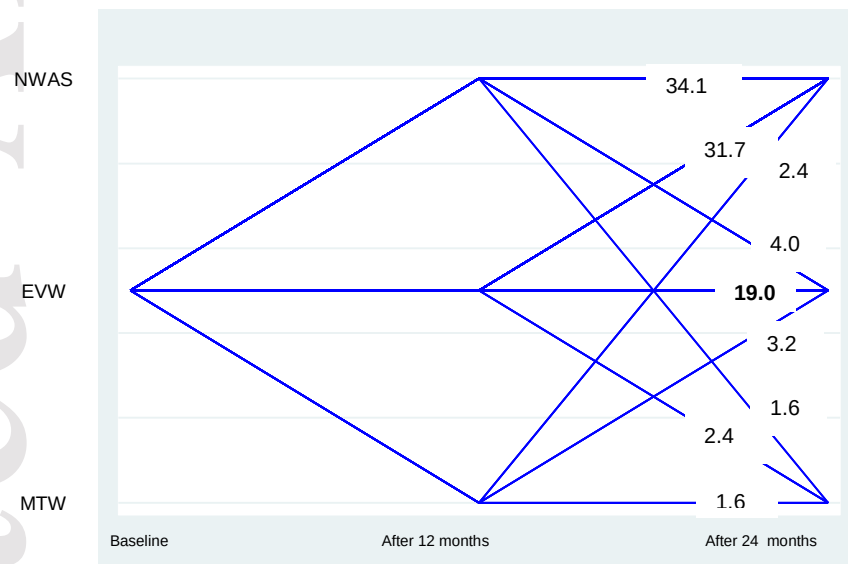
Abbreviations: MTW, Multiple-trigger wheeze; EVW, Episodic viral wheeze; NWAS, Non-wheezy airway symptoms

Numbers are n (percentages in parentheses)



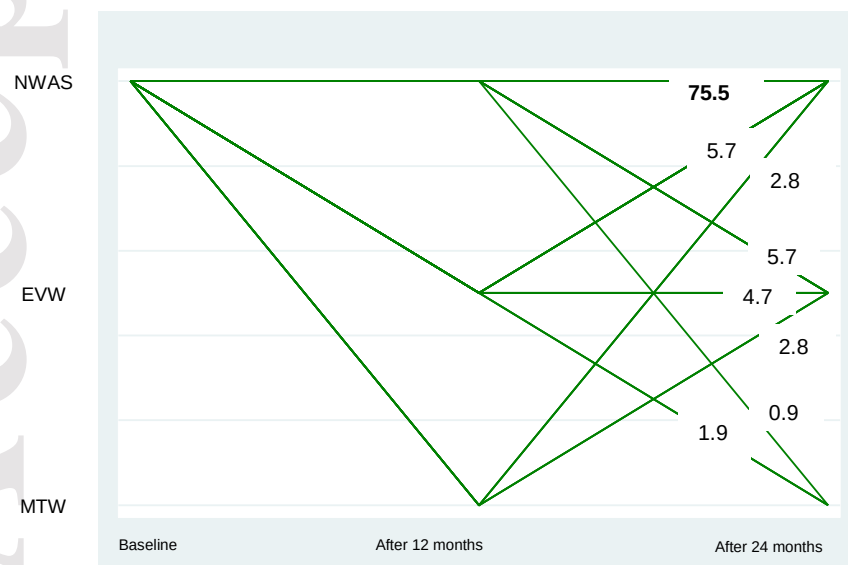
Children with Multiple-trigger wheeze (MTW) at baseline (n=49; 17.4%)

	N	%
MTW-MTW-MTW	10	20.4
MTW-EVW-MTW	1	2.0
MTW-NWAS-MTW	0	0.0
MTW-MTW-EVW	4	8.2
MTW-EVW-EVW	6	12.2
MTW-NWAS-EVW	4	8.2
MTW-MTW-NWAS	8	16.3
MTW-EVW-NWAS	7	14.3
MTW-NWAS-NWAS	9	18.4



Children with Episodic viral wheeze (EVW) at baseline (n=126; 44.8%)

	N	%
EVW-MTW-MTW	2	1.6
EVW-EVW-MTW	3	2.4
EVW-NWAS-MTW	2	1.6
EVW-MTW-EVW	4	3.2
EVW-EVW-EVW	24	19.0
EVW-NWAS-EVW	5	4.0
EVW-MTW-NWAS	3	2.4
EVW-EVW-NWAS	40	31.7
EVW-NWAS-NWAS	43	34.1



Children with Non-wheezing airway symptoms (NWAS) at baseline (n=106; 37.7%)

	N	%
NWAS-MTW-MTW	0	0.0
NWAS-EVW-MTW	2	1.9
NWAS-NWAS-MTW	1	0.9
NWAS-MTW-EVW	3	2.8
NWAS-EVW-EVW	5	4.7
NWAS-NWAS-EVW	6	5.7
NWAS-MTW-NWAS	3	2.8
NWAS-EVW-NWAS	6	5.7
NWAS-NWAS-NWAS	80	75.5

Figure 2 shows the (in)stability of different phenotypes of preschool children after 12 and 24 month follow-up (n=281)

Abbreviations: MTW, Multiple-trigger wheeze; EVW, Episodic viral wheeze; NWAS, Non-wheezy airway symptoms

The 'red figure' (top) shows that 20% of the children with MTW at baseline had MTW at 12 and 24 months. In total, 49% of all children with MTW at baseline were classified as NWAS at 24 months.

The 'blue figure' (middle) shows that 19% of the children with EVW at baseline had EVW at 12 and 24 months. 68% of all children with EVW at baseline were classified as NWAS at 24 months.

The 'green figure' (bottom) shows that 76% of the children with NWAS at baseline had NWAS at 12 and at 24 months.

Phenotype – based on classification at baseline, at 12 and 24 months	Outcome of asthma at age 6 years			
	Asthma (n=83)	No asthma (n=123)	OR	95% CI
Stable MTW	8 (9.6)	1 (0.8)	14.36	1.73 – 119.08
Stable EVW	10 (12.0)	5 (4.1)	3.59	1.15 – 11.25
Stable mixed wheezing	12 (14.5)	4 (3.3)	5.38	1.63 – 17.83
Non-stable wheezing	39 (47.0)	70 (56.9)	Reference	
Stable non-wheezing	14 (16.7)	43 (35.0)	0.58	0.28 – 1.20

Table 3: Wheezing phenotypes and their risk of asthma at 6 years of age (n=206)

Abbreviations: MTW, Multiple-trigger wheeze; EVW, Episodic viral wheeze; OR=odds ratio; CI = confidence interval

Phenotype definitions: Stable MTW: MTW at all three time points; stable EVW: EVW at all three time points; stable mixed wheezing: MTW or EVW at all three time points (but not stable MTW or stable EVW); non-stable wheezing: NWAS on at least one of the three time points (but not stable non-wheezing); stable non-wheezing: NWAS at all three time points.

Numbers are n (percentages in parentheses)

APPENDIX

APPENDIX I – *Classification of phenotypes*

Classification of phenotypes	Phenotypes		
	Baseline	12 months	24 months
Stable MTW	MTW	MTW	MTW
Stable EVW	EVW	EVW	EVW
Stable mixed wheezing	MTW	EVW	MTW
	MTW	MTW	EVW
	MTW	EVW	EVW
	EVW	MTW	MTW
	EVW	EVW	MTW
	EVW	MTW	EVW
Non-stable wheezing	MTW	NWAS	MTW
	MTW	NWAS	EVW
	MTW	MTW	NWAS
	MTW	EVW	NWAS
	MTW	NWAS	NWAS
	EVW	NWAS	MTW
	EVW	NWAS	EVW
	EVW	MTW	NWAS
	EVW	EVW	NWAS
	EVW	NWAS	NWAS
	NWAS	MTW	MTW
	NWAS	EVW	MTW
	NWAS	NWAS	MTW
	NWAS	MTW	EVW
	NWAS	EVW	EVW
	NWAS	NWAS	EVW
	NWAS	MTW	NWAS
	NWAS	EVW	NWAS
Stable non-wheezing	NWAS	NWAS	NWAS

Table 1 Appendix: Phenotype categories determined at baseline, 12 and 24 months

Abbreviations: MTW, Multiple-trigger wheeze; EVW, Episodic viral wheeze; NWAS, Non-wheezy airway symptoms

Phenotype definitions: Stable MTW: MTW at all three time points; stable EVW: EVW at all three time points; stable mixed wheezing: MTW or EVW at all three time points (but not stable MTW or stable EVW); non-stable wheezing: NWAS on at least one of the three time points (but not stable non-wheezing; stable non-wheezing: NWAS at all three time points.

APPENDIX II

PHENOTYPE AT BASELINE	PHENOTYPES							
	<u>After 12 months</u>							
	Prescription of ICS (n=67)				No prescription of ICS (n=214)			
	MTW	EVW	NWAS	TOTAL (100%)	MTW	EVW	NWAS	TOTAL (100%)
MTW (n=49)	8 (47.1)	6 (35.3)	3 (17.6)	17	14 (43.8)	8 (25.0)	10 (31.3)	32
EVW (n=126)	5 (13.2)	27 (71.1)	6 (15.8)	38	4 (4.5)	40 (45.5)	44(50.0)	88
NWAS (n=106)	2 (16.7)	3 (25.0)	7 (58.3)	12	4 (4.3)	10 (10.6)	80 (85.1)	94

Table 2a Appendix: Phenotype classification at baseline and after 12 months in children with and without use of inhaled corticosteroids (n=281)

PHENOTYPE AT BASELINE	PHENOTYPES							
	<u>After 24 months</u>							
	Prescription of ICS (n=79)				No prescription of ICS (n=202)			
	MTW	EVW	NWAS	TOTAL (100%)	MTW	EVW	NWAS	TOTAL (100%)
MTW (n=49)	8 (38.1)	5 (23.8)	8 (38.1)	21	3 (10.7)	9 (32.1)	16 (57.1)	28
EVW (n=126)	4 (9.5)	17 (40.5)	21 (50.0)	42	3 (3.6)	16 (19.0)	65 (77.4)	84
NWAS (n=106)	3 (18.8)	2 (12.5)	11 (68.8)	16	0 (0.0)	12(13.3)	78 (86.7)	90

Table 2b Appendix: Phenotype classification at baseline and after 24 months in children with and without use of inhaled corticosteroids (n=281)

Abbreviations: EVW, Episodic viral wheeze; MTW, Multiple-trigger wheeze; NWAS, Non-wheezy airway symptoms; ICS, inhaled corticosteroids

Numbers are n (percentages in parentheses) unless stated otherwise

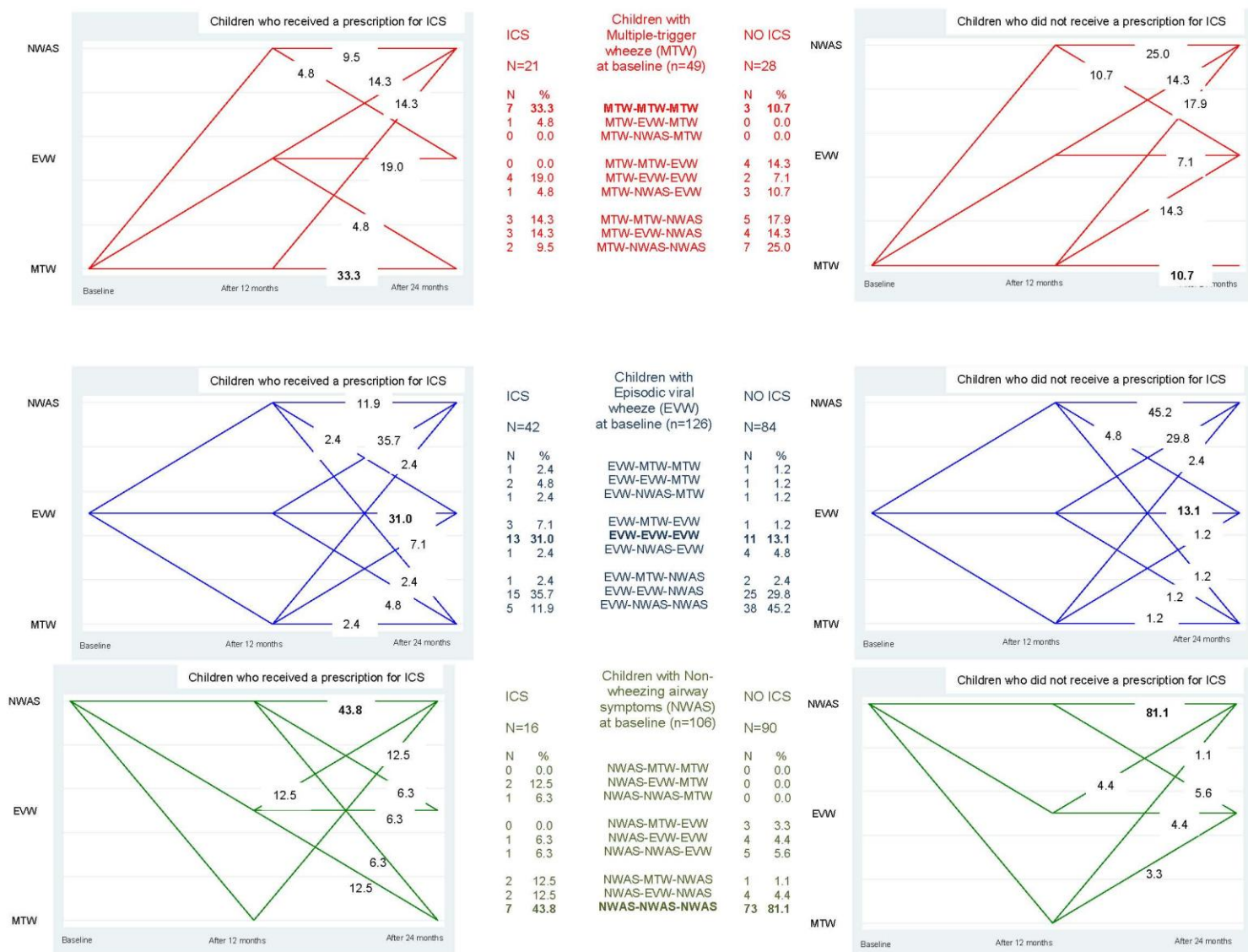


Figure 1 Appendix shows the (in)stability of different phenotypes of preschool children after 12 and 24 month follow-up. In addition, for each phenotype the graphs show which children received a prescription of ICS (left figure) and which children did not receive ICS (right figure) during the 24 month follow-up period (n=281).

Abbreviations: MTW, Multiple-trigger wheeze; EVW, Episodic viral wheeze; NWAS, Non-wheezy airway symptoms; ICS, inhaled corticosteroids

The 'red figure' shows all children with MTW at baseline. The 'blue figure' shows all children with EVW at baseline. The 'green figure' shows all children with NWAS at baseline. The figures show that children with MTW, EVW and NWAS used ICS in 42.9% (21/49), 33.3% (42/126) and 15.1% (16/106) of the cases, respectively during the 24 month follow-up period.

Appendix III

Phenotype – based on classification at baseline, at 12 and 24 months	Asthma (n=83)	No asthma (n=123)	Outcome of asthma at age 6 years	
			OR (95% CI)	
Ever MTW	29 (34.9)	23 (18.7)	3.87	1.72 – 8.75
Never MTW	40 (48.2)	57 (46.3)	2.16	1.04 – 4.45
Stable non-wheezing	14 (16.9)	43 (35.0)	Reference	

Table 3 Appendix: Wheezing phenotypes and risk of asthma at 6 years of age (n=206)

Abbreviations: MTW, Multiple-trigger wheeze; EVW, Episodic viral wheeze; OR=odds ratio; CI = confidence interval

Ever MTW, defined as MTW at baseline or after 12 & 24 months; Never MTW, defined as EVW or NWAS at all three time points (but not stable non-wheezing); Stable non-wheezing, defined as NWAS at all three time points