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Parents know it best: prediction of asthma and lung function by parental perception of early wheezing episodes

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Abstract

Background

Childhood asthma is often preceded by early wheeze. Usually wheezing episodes are recorded retrospectively, which may induce recall bias.

Aims and objectives

The aim of this study was to investigate true positive recall of parent-reported wheeze at 1 year of age, its determinants and its implications for asthma and lung function at 6 years of age.

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Methods

The PASTURE (Protection Against Allergy – Study in Rural Environment) study followed 880 children from rural areas in 5 European countries from birth up to age 6 years. Wheeze symptoms in the first year were asked weekly. At age 6 parent-reported asthma diagnosis was ascertained and lung function measurements were conducted. Correct parental recall of wheeze episodes at the end of the first year was assessed for associations with lung-function, asthma, and the asthma risk locus on chromosome 17q21.

Results

Parents correctly recalled wheeze after the first year in 54% of wheezers. This true positive recall was determined by number of episodes, timing of the last wheeze episode, and parental asthma. Independently from these determinants, true positive recall predicted asthma at age 6 years (odds ratio 4.54, 95%-confidence-interval (CI) [1.75-14.16]) and impaired lung-function (β=-0.62, 95% CI [-1.12; -0.13], p-value=0.02). Associations were stronger in children with asthma risk SNPs on chromosome 17q21.

Conclusion

Correct parental recall of wheezing episodes may reflect clinical relevance of early wheeze and its impact on subsequent asthma and lung function impairment. Questions tailored to parental perception of wheezing episodes may further enhance asthma prediction.

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ABBREVIATIONS

PASTURE Protection against allergy - study in rural environments
ISAAC International Study of Allergy and Asthma in Childhood
AMICS Asthma Multicenter Infants Cohort Study
ALEX Allergy and Endotoxin Study
PARSIFAL Prevention of Allergy Risk factors for Sensitization In children relating to Farming and Anthroposophic Lifestyle study
ATS American Thoracic Society
OR Odds Ratio
HR hazards ratios
β beta-estimates (linear regression)
CI Confidence interval
SNP Single nucleotide polymorphism
IgE Immune globulin E
FEV1 Forced expiratory volume in one second
FVC Forced vital capacity
GLI Global Lung-function Initiative
LPS lipopolysaccharides
IFN- γ Interferon- γ
CH Switzerland
FL Finland
FR France
DE Germany
AT Austria
Chr Chromosome
C-section Cesarean section

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Introduction

With about one in three children affected in the first 3 years of life, wheeze is a common phenomenon in early childhood, implying a substantial burden on children, their caregivers, and healthcare resources (Europrevall) [1, 2].

Suffering from recurrent or persistent early wheeze and exacerbations implies a higher risk for later asthma, airway hyper-responsiveness and decreased expiratory flow [3]. Particularly during the first year of life, wheeze exacerbations might set the stage for impaired airway function and persistent wheeze later in life [4, 5], whereas early diagnosis and intervention might prevent from detrimental lung-sequelae [4, 6]. Low interferon-γ (IFN-γ) levels has been shown to predict subsequent wheeze [7]. Wheeze phenotype classifications are often based on parental reports of age of onset, intensity, and frequency of symptoms combined with suspected trigger factors [8]. Awareness of symptoms, however, varies among parents. Likewise, physicians may interpret reports of wheezing episodes differently, particularly when devices to conduct confirmatory lung-function testing in young children are not available [9-11]. The PASTURE study offered the opportunity to analyse wheeze episodes pro- and retrospectively during the first year of life and to relate them to subsequent development of asthma. The aims of the present analysis were (1) to compare the parent reported wheeze prevalence at 12 months against the cumulative wheeze prevalence derived from weekly diaries from 2 to 12 months without assumption on over- or underestimation, (2) to find determinants affecting parental recall of wheeze episodes, and (3) to estimate the implications of true positive recall at age 12 months on long-term respiratory health outcomes, i.e. asthma and impaired lung function at age 6 years.

Methods

PASTURE is a prospective birth cohort study conducted in five European countries [12]. The study was approved by local research ethics committees, and written informed consent was obtained from the children's parents. Pregnant women living in rural areas were contacted during the last trimester of pregnancy, and 1133 children were recruited shortly after birth

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Questionnaires at 2, 12 and 72 months were based on previously validated questionnaires from the International Study of Allergy and Asthma in Childhood (ISAAC)[13], the Asthma Multicenter Infants Cohort Study (AMICS), the Allergy and Endotoxin Study (ALEX)[14], the Prevention of Allergy Risk factors for Sensitization In children relating to Farming and Anthroposophic Lifestyle study (PARSIFAL)[15] and the American Thoracic Society (ATS)[16].

Information on wheezing episodes was collected via weekly diaries from the 8th until the 53rd week of life using the question "Within the last seven days, has your child had wheezing or whistling sounds while breathing?" meaning a "sound coming out of the breast, not from the nose" [17]. In the 12-month questionnaire, parents were asked how often their child had wheezed since the last home visit at age two months. Possible answer categories were *"never"*, *"less than once a month"*, *"once a month"* and *"at least twice a month"*. The four answer categories were dichotomized; children with any wheeze were classified as wheezers, children whose parents answered "never" were classified as non-wheezers.

Because of prospective and timely collection of data we used wheeze in the diaries as reference. The retrospectively collected information on wheeze using the 12-month questionnaires was then categorized for true positive, true negative, false positive, and false negative recall (Table E1).

Asthma was defined as either parent-reported physician's diagnosis of asthma once or parent-reported physician's diagnosis of obstructive bronchitis more than once when retrospectively asked in the 6 year follow-up questionnaire.

Atopy was defined as inhalant sensitization as previously classified by a latent-class approach over the first 6 years [18].
Lung function measurements were conducted at age 6 years as previously validated [19]. FEV1/FVC z-score transformation based on GLI (Global Lung-function Initiative) equations [20, 21]. Genotyping included previously reported asthma risk alleles on chromosome 17q21 [17]. Interferon-γ (IFN-γ) production at 12 months was measured after 24 hours of incubation of peripheral blood mononuclear with lipopolysaccharides [22].

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Statistical analyses were performed using R 3.3.2 (R Core Team, 2016). Mutually adjusted logistic regression models for true positive recall (versus false negative recall) and asthma, respectively, were established via backward elimination with level-of-entry<0.2 and level-of-stay<0.05.

After log-transformation and subtraction from the maximum value, IFN-γ levels were left-censored. Therefore associations with true positive recall were assessed by Kaplan-Meier-Estimation and Cox proportional hazards-models after confirmation of the proportional hazards-assumption by the Schönefeld Residuals-test. Estimates are reported as odds ratios (ORs), hazards ratios (HR), or beta-estimates (β) with corresponding 95% confidence intervals (CIs).

Results

Of all 1133 children primarily included in the PASTURE birth cohort, 880 children (78 %) had diary data on wheeze events from the 8th until the 53rd week of life and information on asthma status until six years of age and were thus included in the analysis population (Figure E1). Among the included children (n=880) there was a slight predominance of farm exposure, longer duration of breastfeeding, and parental education, whereas smoking during pregnancy was less common (Table E2). Within the analysis population, 611 children completed lung function measurements (Table E3).

The weekly prevalence of wheeze ranged between 1% and 3% and peaked at about 42 weeks; the proportion of concomitant rhinitis increased marginally (Figure 1). The cumulative incidence of wheeze until the first birthday was 35%. In 167 of all 307 children wheezing ever during the first year (54%), parents recalled wheeze symptoms at the end of the first year correctly. Parents of 140 children (46%) did not recall wheeze episodes in the preceding year in the questionnaire at 12 months though they had reported episodes in the diaries (Figure 2A).

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Determinants of true positive recall were parental asthma, total number of wheeze episodes within the first year of life, and a short time lag between completion of the one-year-questionnaire and the last wheeze episode (Table 1). Average duration of wheeze episodes was 4.2 days with no significant difference between children with true positive recall and children with false negative recall (Wilcoxon-test, p=0.37). Sensitivity analyses stratifying for sex and for concomitant rhinitis or excluding Austria (because of no case of asthma) supported the determinants of true positive recall albeit at varying effect size (Table E4). Medication for wheeze was reported only occasionally (2.7 %) including antibiotics (n=15), mucolytics (n=9), inhalant corticosteroids (n=4), and other drugs (n=2) with a non-significant association with true positive recall (OR=1.77, CI 95% [0.59-5.32]).

Asthma was reported in 70 children at age 6 (8.0%). When used as a cumulative measure, the yearly-asked question on asthma led to an overestimation of the asthma prevalence with 21.7% and was not considered for further analyses.

In the group with true positive recall, the risk of asthma was about 4-fold increased as compared to children without wheeze or false negative recall (Figure 2B). In general, the association of asthma with true positive recall (OR=3.45 [1.59-7.51]) was stronger as the association of asthma with any wheeze in the first year in the diaries, i.e. true positive and false negative recall combined (OR=2.89 [1.76-4.79]).

The association of true positive recall and asthma was neither explained by number of wheeze episodes (OR=1.06 [0.95-1.20]) nor by time lag from the last wheeze episode (OR=0.98 [0.94-1.03]). Besides true positive recall, only parental hay fever and being carrier of the asthma risk allele on chromosome 17q21 represented by the SNP rs7216389 emerged as additional asthma-predicting factors in our analysis population (Table 2). Associations were similar across the study centers (Table E5). The association of true positive recall with subsequent asthma was restricted to carriers of the17q21 risk allele (Figure 3). When considering this interaction from a different perspective, association of asthma risk and risk genotype was only seen in children with true positive recall of wheeze (OR=2.46 [0.95-6.39]), whereas asthma risk was unrelated to genotype in children with false negative recall (OR=1.07 [0.12-9.75]).

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Moreover, true positive recall was strongly associated with impaired lung function as determined by the FEV1/FVC-ratio at age 6 years (ß = -0.62 [-1.12; -0.13], Figure 4) independently from parental history of atopy (Table E6).

Children with true positive recall produced about 50% higher IFN-γ levels upon *in vitro* stimulation with LPS as compared to children with false negative recall (p=0.04, Figure E2).

Discussion

When asked prospectively by weekly diaries, about one third of the parents reported at least one wheeze episode in their children during the first year of life; when asked retrospectively at 12 months, however, only half of them remembered those episodes at all. The parental ability to recall those wheezing episodes in their infant's first year of life correctly was related to parental history of asthma, number of wheeze events during that one-year-period and only a short time lag since the last wheeze episode. In turn, true positive recall was associated with a higher risk of subsequent asthma and impaired lung function as compared to false negative recall. The association of true positive recall with asthma was restricted to the asthma risk genotype encoded on 17q21.

Obviously parental observations on wheeze are susceptible to recall bias and vary with respect to disease severity, compliance, and socioeconomic background of the study participants [3, 13, 23]. In the current setting, however, we demonstrate that recall bias can be informative in itself.

The reasons why parents recall wheeze episodes in their children differently may include previous knowledge and experience, cautiousness, health perception, and possibly the impressiveness or severity of the episode. Evidently, parents suffering from asthma themselves are more attentive to wheeze events in their children and might more likely recall wheezing episodes. Moreover, children with a family history of asthma might be affected by more serious wheeze events, which may present more impressively to any observer [24-26]. Likewise, a higher number of total

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wheeze events during the first year of life and a short time lag from the last episode may enhance parental recall.

Despite the rather unspecific questions on wheeze episodes, a strong association of true positive recall with subsequent asthma emerged as the main result of this analysis. This association was independent of all the above determinants for true positive recall, thereby suggesting that parents anticipate subsequent chronic disease from the way the wheeze episodes present. Unfortunately, more detailed information on wheeze episodes was not collected, thus we do not know why these episodes were kept in memory. At least, the mere duration of episodes and usage of wheeze medication within the first year were unrelated to true positive recall though reporting of medication was rather low and possibly incomplete.

The predictive value of wheeze episodes in the first year of life has previously been attributed to the asthma risk genotype encoded on chromosome 17q21, whereas in the non-risk genotype early wheeze was unrelated to subsequent asthma [17, 27]. The current analysis refines this finding insofar as the interaction of genotype and early wheeze is restricted to wheeze episodes that parents recall. Children whose parents forgot wheeze episodes resembled non-wheezing children in their absent association of asthma and genotype. Vice versa, genetically determined more severe forms of asthma may manifest early in life with conspicuous symptoms. Accordingly, genetic testing for the asthma risk genotype on chromosome 17q21 might be advisable in children presenting with remarkable wheezing episodes.

Functionally, the asthma risk genotype on chromosome 17q21 has been implied in enhanced susceptibility to virus infections and impaired antiviral immunity [28]. The latter might also be reflected by lower IFN-y levels upon stimulation with lipopolysaccharides as seen in children with true positive recall. Moreover, wheeze often occurred concomitantly with rhinitis; thus, also the frequency of wheeze episodes may indicate a partially compromised immune system [7, 29].

The strong association of true positive recall with asthma was paralleled by the association of true positive recall with a decreased FEV1/FVC ratio measured at age 6 years. This may indicate that children with more severe wheeze symptoms are al-

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ready prone to deteriorating lung function later in life through incompletely healed injuries of pulmonary tissue or alteration of lung epithelial cell growth [4-6]. Alternatively, causation might be reverse, and children born with compromised airway function might present with more severe symptoms [30].

Current scientific knowledge favours early diagnosis and early medical treatment of asthma symptoms. This may enhance lung growth from the beginning, mitigate progression towards asthma, prevent from lung function impairment, and thus improve quality of life in the long run [6, 31]. However assessment of lung function in very young children is limited or can only be conducted in an incomplete manner [6], thus clinicians often have to rely on basic physical examination, family history, and parental report of symptoms.

Though the quality of data collected via weekly diary questionnaires filled by parents is considered rather high and has been validated against the gold standard of medical records [32], we admit that the mere question on wheeze episodes as asked in the PASTURE study seems rather unspecific. Furthermore, a proper distinction between separate and ongoing wheeze episodes over several weeks was not possible based on the way of questioning within the weekly diary reports. These drawbacks might have led to an underestimation of the predictive value of parental report. Thus, in the future more detailed questions should be included in research questionnaires and standardized history taking.

Another strength of the PASTURE study besides its longitudinal study design is the involvement of children from 5 different European countries, well depicting a profile of distinct European lifestyles. The associations of parental wheeze recall and later asthma were rather consistent over all study centers (I^2<10%) and thus demonstrate reliability and generalizability of our results across various regions of Europe.

This is remarkable since cross-cultural validity and comparability are hampered by language differences, and the terminology of wheeze in non-Anglophone countries might be difficult. In the German language, e.g., there is not even a colloquial term for “wheezing” [33]. Likewise, German-speaking physicians are somewhat reluctant to diagnose “asthma”; among others, they prefer the diagnosis “obstructive bronchi-

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tis". This led to the definition of asthma as a physician's diagnosis of asthma or recurrent obstructive bronchitis in the ISAAC study [13]. Originally this definition was established and validated against cold-air provocation in school-children [34], whereas in a prospective study such as PASTURE it would lead to overestimation of the asthma prevalence when used as a cumulative variable. This phenomenon again suggests that forgetting early episodes of obstructive bronchitis until school age seems justified; oblivion may transport unconscious information.

In conclusion, our findings suggest that parents intuitively notice clinically relevant wheeze episodes in their children. This perception might contribute to a better prognosis of asthma and impaired lung-function later on. Conversely, wheeze episodes without long lasting consequences are more likely forgotten. Parents might therefor be advised not to bother much about mild wheeze episodes. Concurrently, continuous monitoring may run the risk of capturing irrelevant episodes and thus unsettling parents unnecessarily. Nevertheless, history taking should include more detailed questions on alarming wheeze episodes, as it could help identifying children who might benefit from immediate treatment, reduce need for emergency treatment, and enhance quality of life. Ultimately, this analysis demonstrates the value of skilful listening to parents reporting their children's symptoms.

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Tables

Table 1: Determinants of true positive recall of wheeze episodes in children with wheeze in the first year as recorded in diaries (n=307)

Determinants	Crude odds ratio	Adjusted odds ratio
Concomitant rhinitis	0.91 [0.50-1.64]	-
Timing of last wheeze episode (in weeks of age)	1.05 [1.03-1.07]	1.04 [1.01-1.06]
Number of wheeze episodes	1.49 [1.26-1.76]	1.38 [1.17-1.65]
Average duration of wheeze episodes (in days)	0.91 [0.74-1.12]	-
Number of cough episodes	1.09 [1.03-1.16]	-
Number of rhinitis episodes	1.03 [0.98-1.09]	-
Sex	1.56 [0.99-2.47]	-
Farming status	0.75 [0.48-1.18]	-
Mode of birth	1.61 [0.8-3.23]	-
Breastfeeding (>6 months)	1.12 [0.72-1.76]	-
Smoking in pregnancy	1.01 [0.53-1.91]	-
Older siblings	1.18 [0.73-1.91]	-
Parental hay fever	1.09 [0.69-1.71]	-
Parental asthma	2.3 [1.24-4.27]	2.46 [1.27-4.95]
Parental atopic eczema	1.1 [0.5-2.4]	-
Parental atopy	1.32 [0.83-2.08]	-
Parental education	1.06 [0.67-1.67]	-
Having pets in the 4th year	0.71 [0.43-1.19]	-
Day care attendance in the first 3 years	1.44 [0.46-4.55]	-

Odds ratios are given with 95%-confidence intervals. For crude models, only determinants with p-values <0.2 are listed. Adjusted models were established by backward elimination.

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Table 2: Associations of true positive recall of wheeze with an asthma diagnosis (n=880)

Determinants	Crude odds ratio	Adjusted odds ratio
True positive recall	3.45 [1.59-7.51]	4.54 [1.75-14.16]
Sex	2.21 [1.31-3.74]	-
Farmer	0.56 [0.34-0.93]	-
Parental asthma	3.94 [2.3-6.73]	-
Parental hay fever	3.11 [1.83-5.3]	2.86 [1.23-7.09]
Parental atopic eczema	4.08 [1.5-11.13]	-
Parental atopy	2.83 [1.61-4.97]	-
Chr 17q21 (rs7216389)	1.68 [1.12-2.52]	1.92 [1.05-3.61]

Odds ratios are given with 95%-confidence intervals. For crude models, only determinants with p-values <0.05 are listed. Adjusted models were established by backward elimination.

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Legends to figures

Figure 1: Wheeze weekly in the 1st year of life

Figure 2: True positive recall of wheeze episodes and subsequent asthma

Information on wheeze based on weekly diary questionnaires or on the questionnaire at 1 year. True negatives are defined as no wheeze reported by both instruments. False positive means wheeze reported in the questionnaire at 1 year only. False negative recall means wheeze reported only in weekly diaries but not recalled in the questionnaire at 1 year. True positive recall are defined as wheeze reported by both instruments. The total number of children with data provided by both instruments was 880.

Figure 3: Asthma risk stratified for true positive recall and genotypes (CC, CT, TT) of asthma risk allele (rs7126389) on chromosome 17q21

Figure 4: Lung function (FEV1/FVC) in children with false negative recall and true positive recall

Given is a standard box-and-whiskers plot with boxes representing the inner quartiles and whiskers extending to maximum and minimum values within 1.5 times the interquartile range.

*P-values are based on an Anova and a post-hoc t-test.

** FEV1/FVC z-score based on GLI (Global Lung-function Initiative) equations.

Figure E1: Selection of the study population and timeline of the PASTURE study up to age 6 years

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Figure E2: IFN-γ levels in children with true positive recall and false negative recall

*to achieve normal distributed, left censored IFN-y values, original values were first log-tranformed and then subtracted from max. IFN-y

HR=0.68, CI 95% [0.46-0.98]

Proportional hazards assumption was previously confirmed (Schönefeld Residuals Test, p-value= 0.51)

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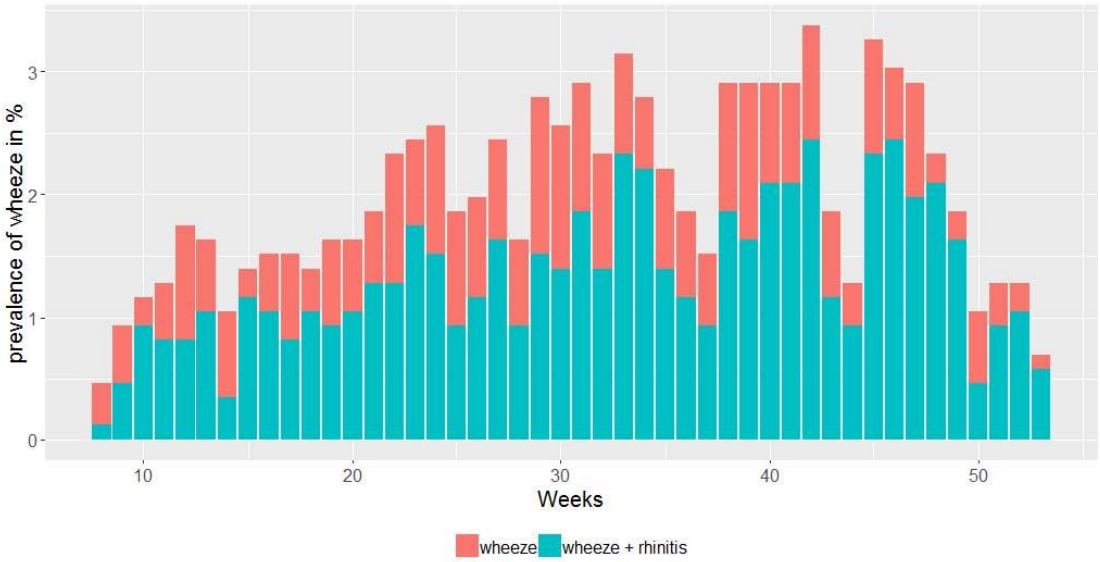

Figure 1: Wheeze weekly in the 1st year of life

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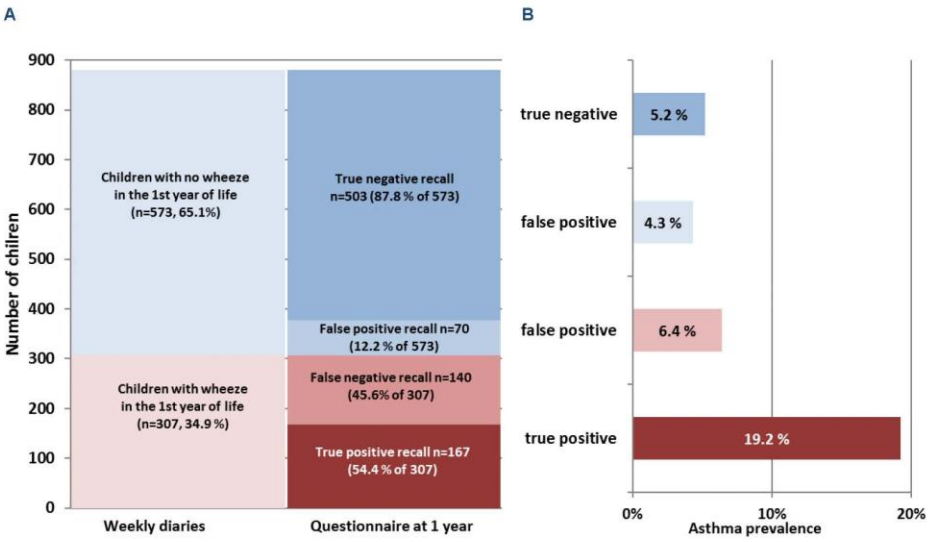

Figure 2: True positive recall of wheeze episdodes and subsequent asthma

Information on wheeze based on weekly diary questionnaires and on the questionnaire at 1 year. True negatives are defined as no wheeze reported in both instruments. False positive means wheeze reported in th questionnaire at 1 year only. False negative recall means wheeze reported only in weekly diaries but not recalled in the questionnaire at 1 year. True positive recall is defined as wheeze reported in both instruments. The total number of children with data provieded by both instruments was 880.

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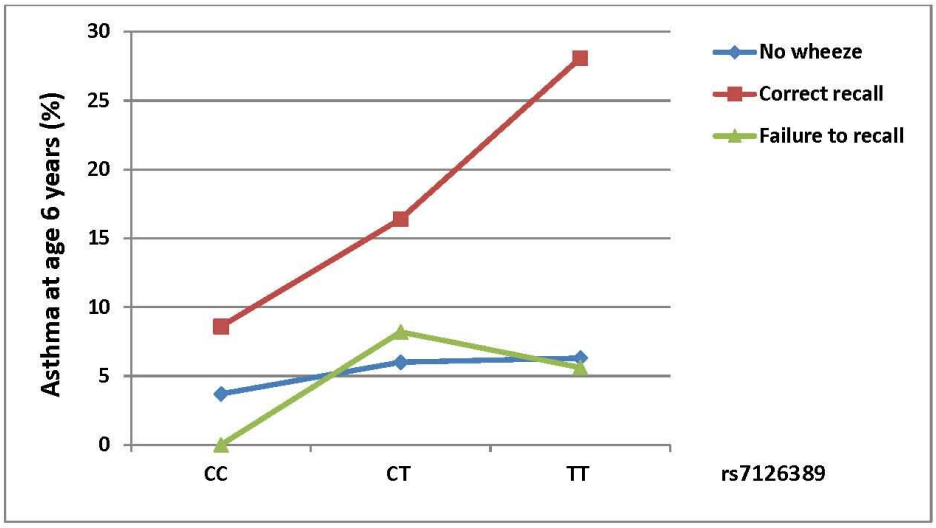

Figure 3: Asthma risk stratified for correct recall and genotypes (CC, CT, TT) of asthma risk allele (rs7126389) on chromosome 17q21

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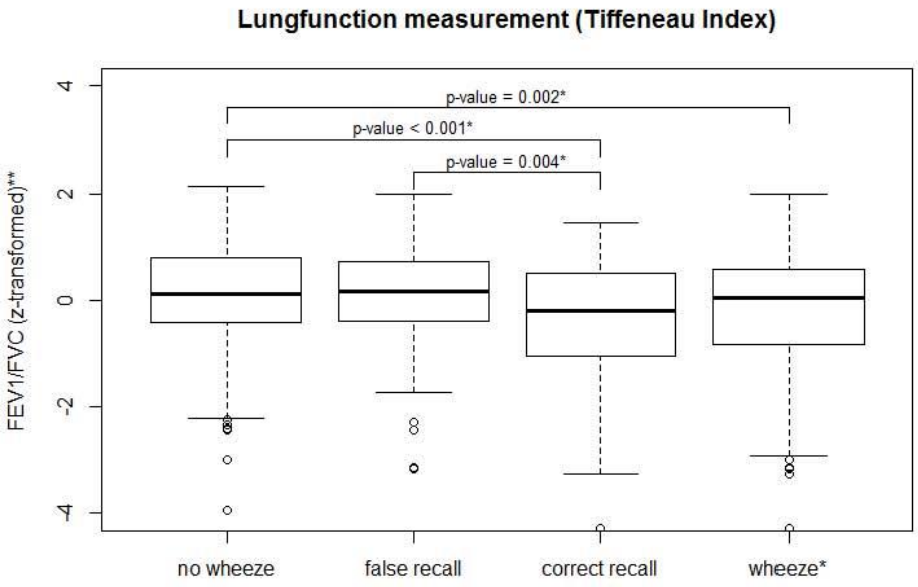

Figure 4: Lung function (FEV1/FVC) in children with false negative recall and true positive recall

Given is a standard box-and-whiskers plot with boxes representing the inner quartiles and whiskers extending to maximum and minimum values within 1.5 times the interquartile range.

*P-values are based on an Anova and a post-hoc t-test.

** FEV1/FVC z-score based on GLI (Global Lung-function Initiative) equations.

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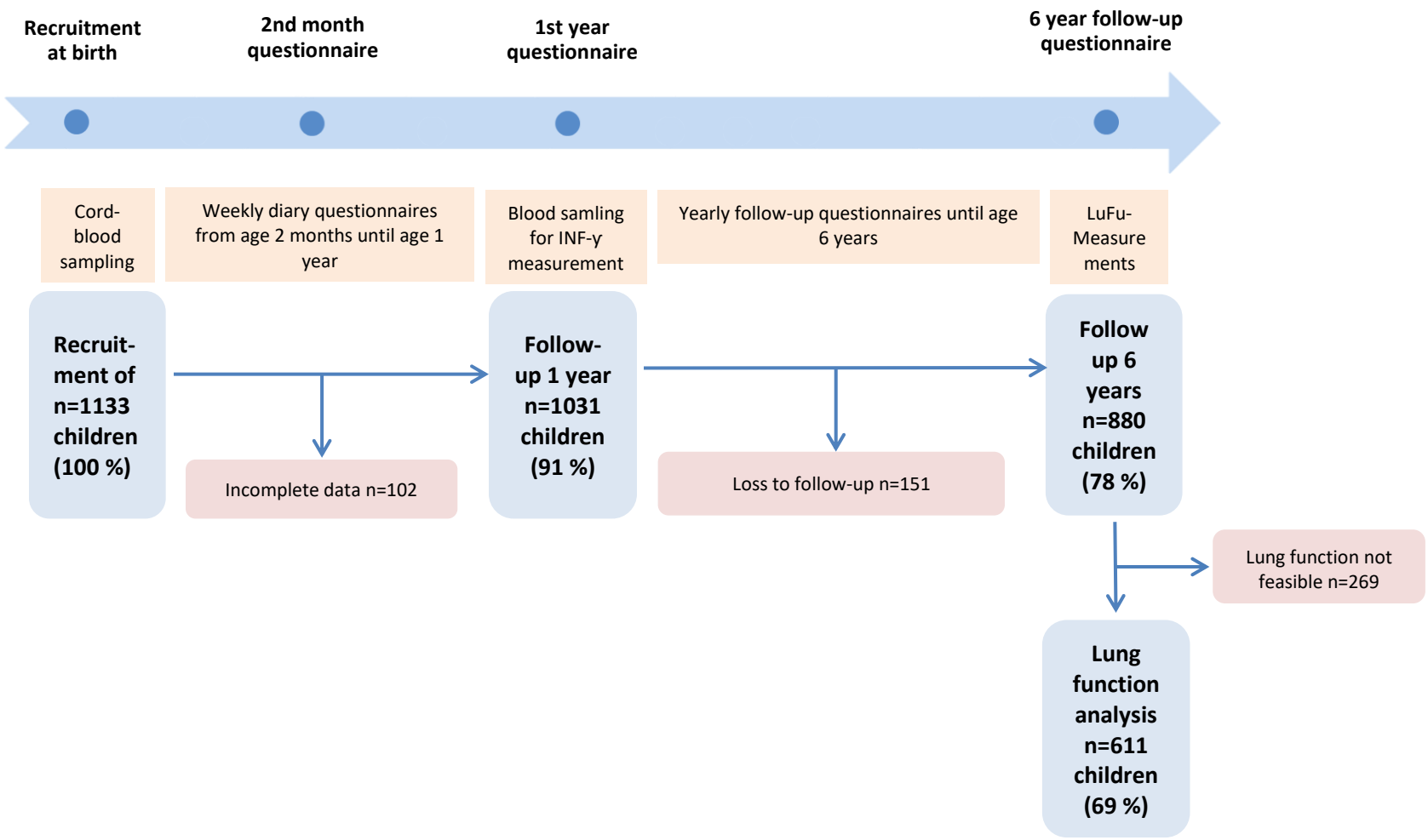

Figure E1: Selection of the study population and timeline of the PASTURE study up to age 6 years

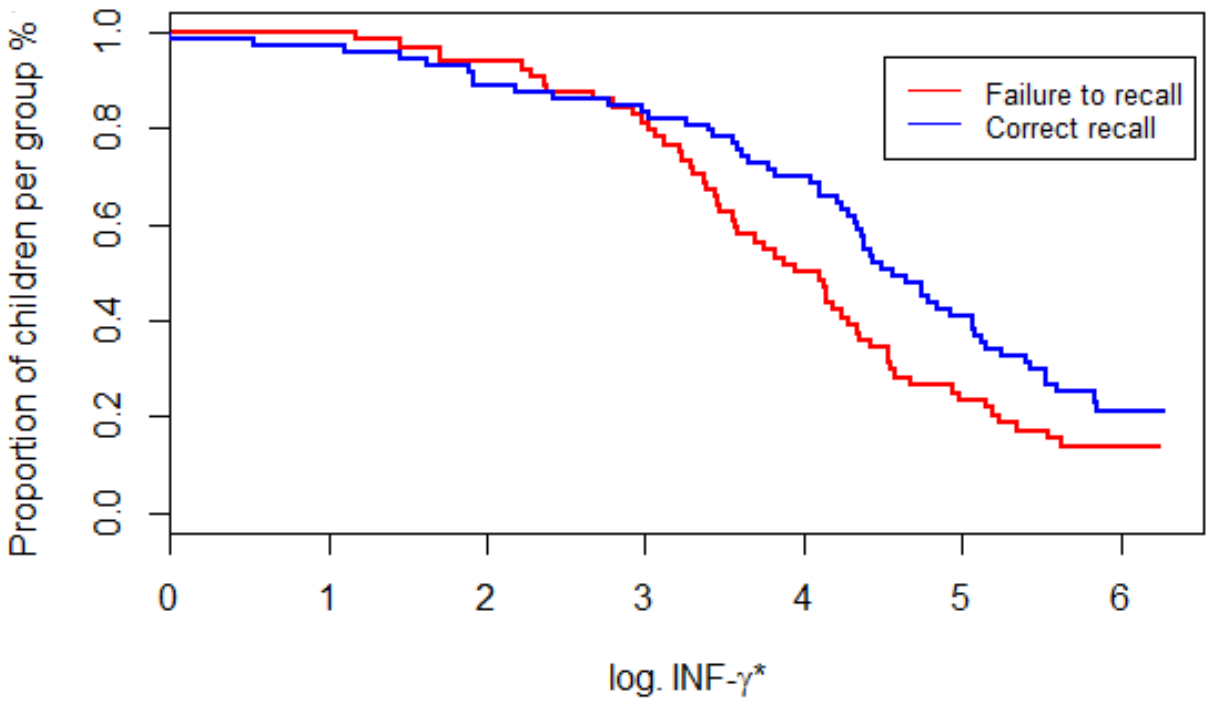

Figure E2: INF-γ levels in children with correct recall and failure to recall
*to achieve normal distributed, left censored INF-y values, original values were first log-tranformed and then subtracted from max. INF-y.
HR=0.68, CI 95% [0.46-0.98] Proportional hazards assumption was previously confirmed (Schönefeld Residuals Test, p-value= 0.51)

Table E1: Definition of true and false recall

Wheeze prospectively recorded in diaries	Wheeze retrospectively recorded at 12 months	Recall	Coding for analysis
present	present	True positive	1
present	absent	False negative	0
absent	present	False positive	NA
absent	absent	True negative	NA

Individuals with wheeze episodes absent in the diaries were coded as ‘not available’ (NA) in statistical analyses.

Table E2: Individuals included and not included in the analysis population

Variable	Included (n = 880)[#]	Not included (n = 253)[#]	p-value[°]
Asthma	70 (8.0%)	8 (16.7)	0.06
Atopy	154 (21.6%)	9 (16.7%)	0.49
Male sex	450 (51.1%)	110 (52.4%)	0.81
Living on a farm	429 (48.8%)	101 (39.9%)	0.02
High parental education	460 (52.8%)	102 (45.9%)	0.08
Parental asthma	131 (15.0%)	34 (16.1%)	0.76
Parental hay fever	390 (44.5%)	101 (45.7%)	0.80
Parental atopy	472 (53.8%)	119 (53.6%)	1.0
Older siblings	557 (63.3%)	163 (64.4%)	0.80
Breastfeeding at least 6 months	495 (56.2%)	74 (45.1%)	0.01
Mode of birth (C-section)	164 (18.8%)	28 (13.4%)	0.08
Smoking during pregnancy	105 (12.0%)	53 (21.0%)	<0.001
Day care	33 (4.9%)	5 (4.9%)	0.85
Pets at home	240 (28.1%)	38 (28.1%)	0.59

[#]Variable comparison among those with information on the respective variable

°p-value from fisher's exact test

Table E3: Individuals included and not included in the lung-function measurements at age 6 years among all 880 children of the analysis population

Variable	Included (n = 611)[#]	Not included (n = 269)[#]	p-value°
Asthma	54 (8.8%)	16 (5.9%)	0.19
Atopy	121 (19.8%)	33 (12.3%)	0.01
Male sex	327 (53.5%)	123 (45.7%)	0.04
Living on a farm	298 (48.8%)	131 (48.7%)	1.0
High parental education	328 (54.0%)	132 (50.0%)	0.31
Parental asthma	100 (16.5%)	31 (11.6%)	0.08
Parental hay fever	280 (46.0%)	110 (41.0%)	0.2
Parental atopy	341 (56.0%)	131 (48.9%)	0.06
Older siblings	398 (65.1%)	159 (59.1%)	0.1
Breastfeeding at least 6 months	332 (54.3%)	163 (60.6%)	0.1
Mode of birth (C-section)	109 (18.0%)	55 (20.6%)	0.41
Smoking during pregnancy	77 (12.6%)	28 (10.4%)	0.42
Day care	23 (5.0%)	10 (4.6%)	0.96
Pets at home	161 (27.1%)	79 (30.5%)	0.34

[#]Variable comparison among those with information on the respective variable

°p-value from fisher's exact test

Table E4: Determinants of true positive recall of wheeze (stratified for sex, wheeze with and without concomitant rhinitis and all centers and all centers but Austria)

Determinants	OR [95%CI]	OR [95%CI]
	Boys (n=174)	**Girls (n=133)**
Parental asthma	1.85 [0.74-4.89]	3.64 [1.34-11.04]
Timing of last wheeze episode (in weeks of age)	1.05 [1.01-1.09]	1.02 [0.98-1.05]
Number of wheeze episodes	1.65 [1.27-2.28]	1.18 [0.97-1.51]
	Children with wheeze and concomitant rhinitis (n=243)	**Children with wheeze only (n=54)**
Parental asthma	3.02 [1.46-6.55]	1.11 [0.11-7.40]
Timing of last wheeze episode (in weeks of age)	1.04 [1.01-1.07]	1.05 [1.00-1.12]
Number of wheeze episodes	1.42 [1.20-1.73]	1.18 [0.75-1.01]
	All centers (n=307)	**All centers but Austria (n=257)**
Parental asthma	2.46 [1.27-4.95]	2.30 [1.15-4.77]
Timing of last wheeze episode (in weeks of age)	1.04 [1.01-1.06]	1.04 [1.01-1.06]
Number of wheeze episodes	1.38 [1.17-1.65]	1.35 [1.15-1.63]

Multiple logistic regression models were used to calculate sex-specific associations between true positive recall and asthma.

Table E5: Associations of true positive recall with asthma and lung-function

Associations of true positive recall of wheeze with an asthma diagnosis – whole study population and separately in study centers			
	OR [CI 95%]	P-value	I² (Inconsistency)
Total study population	3.45 [1.59-7.51]	0.0018	0.0%
Germany	2.48 [0.6-10.21]	0.208	
Austria[#]	(>5.22)	-	
Switzerland	1.85 [0.33-10.42]	0.483	
France	2.06 [0.35-12.02]	0.422	
Finland	6.07 [1.18-31.24]	0.031	
Associations of true positive recall of wheeze with lung-function parameters (Tiffenau Index FEV1/FVC) – whole study population and separately in study centers			
	ß-Estimate [CI 95%]	P-value	I² (Inconsistency)
Total study population	-0.41 [-0.68; -0.13]	0.005	6.74%
Germany	-0.24 [-1.04; 0.29]	0.412	
Austria	-0.95 [-1.60; -0.31]	0.017	
Switzerland	-0.59 [-1.41; 0.14]	0.075	
France	-0.25 [-0.79; 0.17]	0.255	
Finland	-0.10 [-0.71; 0.54]	0.737	

Logistic regression models were used to calculate center-specific associations between wheeze recall and asthma.

[#]in Austria no case of asthma was observed among children with false negative recall; if there were one case, the OR would be 5.22

Table E6: Associations of true positive recall of wheeze with lung-function parameters (Tiffenau Index FEV1/FVC z-score)

Determinants	Crude β-Estimate [95% CI]	Adjusted β-Estimate [95% CI]
True positive recall	-0.41 [-0.68; -0.12]	-0.62 [-1.12; -0.13]
Sex	-0.38 [-0.47; -0.16]	-
Parental asthma	-0.28 [-0.49; -0.07]	-
Parental hay fever	-0.12 [-0.28; 0.04]	-
Parental atopic eczema	-0.5 [-0.80; -0.21]	-0.51 [-1.01; -0.01]
Parental atopy	-0.18 [-0.34; -0.02]	-

Only determinants with crude p-values <0.2 are listed. β-estimates are given with 95%-confidence intervals (CI). Adjusted models were established by backward elimination. FEV1/FVC z-score based on GLI (Global Lung-function Initiative) equations.

Online supplement

Methods

Study design and population

The PASTURE project is a prospective birth cohort study which started in 2002. The prior study aim was to assess influencing factors of rural and farming environment on the development of asthma and allergies in children. The study was conducted in five European countries; Austria, Finland, France, Germany and Switzerland. Local research ethics committees in each country approved the study; written informed consent was obtained from the children's parents. Detailed information on study design is described elsewhere [1].

Initially pregnant women living in rural areas were contacted during the last trimester of pregnancy. 1133 children matching the inclusion criteria were recruited shortly after birth. Detailed information on the study design are described elsewhere[1]. Children without information on wheeze events during their first year of life (n=104) and who did not complete the follow-up period of 6 years (n=214) were excluded; thus 880 children with sufficient background and health information were included in the following analysis. Lung function measurements were performed in a subgroup of 611 children (Figure 1).

Questionnaires

The questionnaires of the PASTURE study based on items of the International Study of Allergy and Asthma in Childhood (ISAAC) [2], the Asthma Multicenter Infants Cohort Study (AMICS), the Allergy and Endotoxin Study (ALEX) [3], the Prevention of Allergy Risk factors for Sensitization In children relating to Farming and Anthroposophic Lifestyle study (PARSIFAL) [4] and the American Thoracic Society (ATS) [5] questionnaire.

Parents were initially asked to complete a questionnaire at two months age of their child. Thereafter additional information on farm-related exposures and feeding practices, occurring infections, medication and wheezing episodes of the infant was collected via weekly diary questionnaires from the 8th until the 53rd week of life[1]. In the one-year-

questionnaire, parents were asked how often their child had wheezed since the last home visit at age two months. Possible answer categories were *"never"*, *"less than once a month"*, *"once a month"* and *"at least twice a month"*. The four answer categories were dichotomized; children with any wheeze were classified as wheezers, children whose parents answered "never" were classified as non-wheezers.

Thereupon regular questionnaires were handed out the parents up to six years of age. These questionnaires comprised detailed information about current socioeconomic and lifestyle, agricultural exposures and atopic symptoms and further health issues of the child and the family of origin. A physician's diagnosed asthma was enquired in the six-year-questionnaire.

Reference standards and definition of true positive and false negative reporting

Report of wheeze episodes via weekly questionnaires between the 8th and 53rd week of life was regarded as reference standard. Correct recall and failure to recall were defined as followed.

True positive recall: at least one wheezing episode during the child's first year of life reported in the weekly diary questionnaires plus retrospectively correct recall of the wheezing episodes when asked "Since we last visited you, how often has your child had wheezing or whistling" in the one-year questionnaire.

False negative recall: at least one wheezing episode during the child's first year of life reported in the weekly diary questionnaires but no recall of the wheezing episodes when asked "Since we last visited you, how often has your child had wheezing or whistling" in the one-year questionnaire.

Children whose parents did not report any wheeze in the diary questionnaires were subdivided into true negative recall (children with no wheeze ever until the age of 6 years) and false positive recall (children whose parents reported wheeze in the one-year questionnaire).

Asthma was defined as either parent-reported physician's diagnosis of asthma once or parent-reported physician's diagnosis of obstructive bronchitis more than once when retrospectively asked in the 6 year follow-up questionnaire.

Atopy was defined as inhalant sensitization as previously classified by a latent-class approach over the first 6 years [6].

Measurements

Lung function measurements were conducted at 6 years age as previously validated [7]. Forced expiratory volume in one second (FEV1) and forced vital capacity (FVC) were measured. Hence based on GLI (Global lungfunction initiative) [8, 9] equation the z-transformed Tiffeneau Index (ratio of FEV1 and FVC) was calculated for every child. Cord blood was collected and genotyped for previously reported asthma risk alleles on chromosome 17q21 [10] to investigate in possible associations of genetic predisposition and parental ability to recall wheezing episodes. Peripheral blood mononuclear cells were isolated at age 1 year and incubated with LPS (lipopolysaccharides) for 24 hours. Interferon- γ (IFN- γ) production was measured via multiplexed cytometric bead array according to the manufacturer's instructions (BD) in Marburg, Germany [11]. Resulting IFN-γ values were standardized on leukocyte counts.

Statistical Analysis

Comparison of background characteristics between children in the recruitment, the one-year follow-up and the six-year follow-up population were calculated via Fisher's exact test. Mutually adjusted logistic regression models for true positive recall (versus false negative recall) and asthma, respectively, were established via backward elimination with level-of-entry<0.2 and level-of-stay<0.05.

After log-transformation and subtraction from the maximum value, IFN-γ levels were left-censored. Then IFN-γ levels between children with correct recall and failure to recall were compared via Kaplan-Meier-Estimation and Cox proportional hazards models after confirmation of the proportional hazards-assumption by the Schönefeld Residuals-test. Estimates are reported as odds ratios (ORs), hazards ratios (HR), or beta-estimates (β) with corresponding 95% confidence intervals (CIs), respectively. All reported logistic regression models were considered statistically significant with a p-value < 0.05. Statistical analyses were performed using R 3.3.2 (R Core Team, 2016).

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