

1 **Do clinical investigations predict long-term wheeze?**
2 **A follow-up of paediatric respiratory outpatients**

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39 **Running title: Predicting wheeze: a follow-up study** (max. 50 characters)

40 **Do clinical investigations predict long-term outcome?**

41 **A follow-up of paediatric respiratory outpatients**

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43

44 **Abstract (250/250)**

45 **Introduction:** The contribution of clinical investigations to prediction of long-term outcomes
46 of children investigated for asthma is unclear.

47 **Aim:** We performed a broad range of clinical tests and investigated whether they helped to
48 predict long-term wheeze among children referred for evaluation of possible asthma.

49 **Methods:** We studied children aged 6-16 years referred to two Swiss pulmonary outpatient
50 clinics with a history of wheeze, dyspnoea, or cough in 2007. The initial assessment included
51 spirometry, fractional exhaled nitric oxide, skin prick tests, and bronchial provocation tests
52 (BPT) by exercise, methacholine, and mannitol. Respiratory symptoms were assessed with
53 questionnaires at baseline and at follow-up seven years later. Associations between
54 baseline factors and wheeze at follow-up were investigated by logistic regression.

55 **Results:** At baseline, 111 children were examined in 2007. Seven years after baseline, 85
56 (77%) completed the follow-up questionnaire, among whom 61 (72%) had wheeze at
57 baseline, while at follow-up 39 (46%) reported wheeze. Adjusting for age and sex, the
58 following characteristics predicted wheeze at adolescence: wheeze triggered by pets (odds
59 ratio 4.2, 95% CI 1.2-14.8), pollen (2.8, 1.1-7.0), and exercise (3.1, 1.2-8.0). Of the clinical
60 tests, only a positive exercise test (3.2, 1.1-9.7) predicted wheeze at adolescence.

61 **Conclusion:** Reported exercise-induced wheeze and wheeze triggered by pets or pollen
62 were important predictors of wheeze persistence into adolescence. None of the clinical tests
63 predicted wheeze more strongly than reported symptoms. Clinical tests might be important
64 for asthma diagnosis but medical history is more helpful in predicting prognosis in children
65 referred for asthma.

66

67 **Introduction**

68 Asthma is the most prevalent chronic respiratory disease in childhood and adolescence,
69 which leads to many health care visits¹⁻³. Its key symptoms are wheeze, cough, and difficulty
70 breathing, but symptoms vary substantially between individuals and across ages^{1,2}. Some
71 children who present with asthma symptoms continue to have problems later in life, while
72 others do not. Better knowledge of their individual prognoses might affect their follow-up and
73 answer questions of parents in the clinics⁴⁻⁶. Assessing prognosis of asthma symptoms from
74 school age into adulthood and identifying children at high-risk of symptom persistence is
75 challenging⁴.

76 Studies investigating prognosis of asthma or wheeze in school-aged children are conducted
77 with either clinical asthma cohorts or symptomatic children of a population-based cohort⁷.

78 Studies in clinical asthma cohorts have found that lower FEV₁, asthma severity, sensitisation
79 to indoor allergens, eczema, hay fever, skin test reactivity, and bronchial hyper-
80 responsiveness were associated with asthma persistence⁸⁻¹⁰. Studies in population-based
81 cohorts have found that wheeze persistence was predicted by frequent attacks of wheeze,
82 female sex, sensitization to furred animals or house dust mites, rhinitis, and bronchial hyper-
83 responsiveness¹¹⁻¹⁶.

84 For clinical practice, two knowledge gaps remain. First, few studies have examined the
85 prediction of long-term prognosis, but none have done this for school-aged children seen in
86 outpatient clinics for possible asthma. Second, many tests are performed in clinics to
87 diagnose these children, but it is unclear whether these tests predict prognosis more
88 accurately than reported symptoms alone. We determined whether clinical tests in addition
89 to reported symptoms help predict wheeze in adolescence in school-aged children referred
90 for possible asthma.

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95 **Methods**

96 **Study population and study design**

97 Of the 124 children invited, 111 were recruited from the respiratory outpatient clinics of two
98 paediatric hospitals in Switzerland, 84 from St. Gallen and 27 from Basel, who were eligible
99 if they had been referred for evaluation of current wheeze, dyspnoea, or cough. Children
100 with a known chronic respiratory disease such as cystic fibrosis or primary ciliary dyskinesia,
101 or a respiratory tract infection during four weeks prior to the visit were excluded. At baseline
102 in 2007-2008, parents completed a questionnaire and children underwent a set of
103 standardised clinical tests during two different visits within one week as part of the study
104 protocol^{17,18}. At follow-up, seven years after baseline, in 2014 to 2015, we sent a
105 questionnaire to the 12-23 year-old adolescents or young adults (from now on referred to as
106 adolescents) (E-figure 1).

107 Ethical approval was obtained from the local Ethics committee and all parents gave informed
108 consent during the first visit at baseline and by sending back the questionnaire at follow-up
109 (EKSG 07/001).

110

111 **Baseline assessment**

112 The parental questionnaire included ISAAC key questions¹⁹ plus additional questions on
113 type and triggers of respiratory symptoms, atopic symptoms, previous treatments and
114 environmental exposures (Supplementary questionnaire 1 (German, original) and 2 (English,
115 translation)). The study physician reported clinical test results, final diagnosis, and
116 prescribed medication in a uniform way. Physicians diagnosed the children after all clinical
117 tests were done, taking into consideration medical history, clinical examination, and all test
118 results. Vocal cord dysfunction was diagnosed based on medical history, physical
119 examination and normal expiratory curves in spirometry.

120 The baseline assessment consisted of two visits. At the initial baseline visit, children
121 performed spirometry, fractional exhaled nitric oxide (FeNO) measurement, a skin prick test
122 (SPT), bronchial provocation test (BPT) by exercise and, by methacholine. At the second

123 baseline visit, children did a BPT by mannitol. All clinical tests were performed according to
124 published guidelines²⁰⁻²⁴. A detailed description of the test procedures has been published
125 elsewhere^{17,18,25} and is included in the online supplementary material (E-text). Lung function
126 measurements were compared to reference values from Zapletal et al²⁶. We considered the
127 exercise test as positive in the event of a $\geq 15\%$ decrease in the FEV₁ after the exercise
128 challenge test, and the methacholine test as positive when the minimal dose causing a
129 $\geq 20\%$ decrease of FEV₁ was $< 1\text{mg}$ (the provocation dose, PD 20). The mannitol dry powder
130 challenge test was considered as positive when a 15% fall in FEV₁ was measured before a
131 cumulative dose of 635 mg was reached, or when a 10% fall in FEV₁ between two doses
132 was reached. FeNO was measured using the portable NIOX MINO® device, and was
133 considered as positive when FeNO was higher than 26ppb¹⁸. We performed skin prick tests
134 for birch, grass, mugwort, alternaria, cat, house dust mites (*D. pteronyssinus*), and positive
135 and negative controls¹⁸. These allergens cover 95% of inhaled allergens in Switzerland²⁷.
136 The test was considered to be positive if any mean wheal diameter was $\geq 3\text{mm}$.

137

138 **Assessment at follow-up**

139 The follow-up questionnaire was very similar to the baseline questionnaire, but the questions
140 were addressed directly to the adolescents instead of their parents (supplementary
141 questionnaire 3 (German, original) and 4 (English, translation)).

142

143 **Definitions of wheeze and frequent wheeze**

144 We assessed wheeze at follow-up with the question, "Have you had a whistling sound in the
145 chest in the last 12 months?" If a child had had more than three attacks of wheeze in the last
146 12 months, we considered the child to have had frequent wheeze.

147

148 **Statistical analysis**

149 We compared the participants with information at baseline and follow-up to those without
150 follow-up information to test for selection bias, using chi-square test. The participants with

151 information at baseline and follow-up were included in the analysis.
152 We investigated the association between exercise-induced wheeze and a positive exercise
153 test at baseline using the Fisher's exact test, and the Mann-Whitney-U test when looking at
154 the association of reported exercise-induced wheeze and the fall of FEV₁% predicted during
155 the exercise test.

156 We investigated the association between symptoms (table 1) and clinical test results (table
157 2) at baseline with any wheeze and frequent wheeze at follow-up using logistic regression,
158 adjusting for sex and age. For comparison, we repeated the analysis including only children
159 diagnosed with asthma (N=62). We did not consider interactions or a multivariable model
160 because of the sample size. We used STATA software (version 14; College Station, Texas)
161 to analyse the data.

162

163 **Results**

164 **Characteristics of the study population at baseline and at follow-up**

165 Eighty-five (77%) of the 111 children who participated in the baseline study completed the
166 follow-up questionnaire. The median age was 12 years at baseline (range 6-16) and 18 at
167 follow-up (12-23); 60% (51/85) were male. Wheeze was reported by 61 (72%) at baseline,
168 and 7 reported cough without wheeze, 12 (14%) reported exercise-related breathing
169 problems and 5 (6%) reported allergic rhinitis. Among those with wheeze, 27 (44%) had
170 more than three attacks during 12 months prior to the baseline visit (Table 1). Symptoms at
171 baseline were very similar in children who did not take part in the follow-up (E-table 1 online
172 supplementary material). Asthma medication was prescribed at the baseline visit for 71
173 (85%) children, of whom 47 (55%) received inhaled short-acting β 2-agonists (SABA) alone,
174 6 received SABA and inhaled corticosteroids (ICS), and 18 received long-acting β 2-agonists
175 (LABA) and ICS. At follow-up, 39 (46%) participants reported wheeze of whom 30 had more
176 than 3 attacks during the last year. At follow-up, 44 adolescents (52%) reported using

177 inhalers, including 21 using SABA alone, 2 using SABA and ICS, and 21 using LABA and
178 ICS (Table 1).

179

180 Table 2 shows the clinical test results and diagnoses at baseline. All tests were completed in
181 at least 90% of the children. The main reason for not completing a BPT was exhaustion^{17,18}.

182 For the 78 children who completed the BPT by methacholine at baseline, the test was

183 positive in 76% and the median provocation dose was 0.14mg. Eighty-two completed the

184 BPT by mannitol, of whom 28% tested positive. The median provocation dose was 635 mg.

185 Of the 76 children who completed the BPT by exercise, the median fall of FEV₁ was 8%

186 predicted. The test was positive ($\geq 15\%$ decrease in the FEV₁) in 18 (24%) children. SPT

187 was positive in 33 (39%) children and FeNO was positive in 35 (41%). Doctors diagnosed 62

188 (73%) children with asthma or episodic viral wheeze. The other children were mostly

189 diagnosed with cough not due to asthma or vocal cord dysfunction.

190 At baseline, self-reported exercise-induced wheeze was associated with a positive exercise

191 test ($p=0.022$, E-table 2).

192

193 **Baseline factors associated with wheeze and frequent wheeze at follow-up**

194 Four respiratory symptoms and one clinical test at baseline were associated with *any*

195 *wheeze* at follow-up. Of the reported symptoms, frequent wheeze (>3 attacks) (OR 2.86,

196 95% CI 1.10-7.43), exercise-induced wheeze (3.07, 1.19-7.96), wheeze triggered by pets

197 (4.22, 1.21-14.76), and wheeze triggered by pollen (2.78, 1.11-6.98) were associated with

198 wheeze at follow-up. For the clinical tests, only a positive exercise test was significantly

199 associated with wheeze seven years later (3.20, 1.05-9.70). Results remained very similar

200 after adjusting for age and sex (Table 3). When we repeated the analysis for children

201 diagnosed with asthma (N=62), we found mostly comparable results (E-table 3). However,

202 associations tended to be less strong (lower odds ratios) in particular for exercise induced

203 wheeze (1.79, 0.58-5.48) and positive exercise test (2.00, 0.63-6.39)

204

205 Two respiratory symptoms were associated with *frequent wheeze* at follow-up. These were
206 exercise-induced wheeze (OR 3.05, 95% CI 1.07-8.67) and wheeze triggered by pets (3.79,
207 1.15-12.48; E-table 4). None of the clinical test results were associated with frequent wheeze
208 at follow-up.

209

210 **Discussion**

211 Among school-aged children referred to a respiratory outpatient clinic for evaluation of
212 wheeze, cough, or dyspnoea, 46% reported wheeze seven years later. Reported exercise-
213 induced wheeze and wheeze triggered by pets or pollen at baseline predicted wheeze at
214 follow-up. Of the clinical tests, only a positive exercise challenge test predicted wheeze at
215 follow-up, but no more strongly than reported exercise-induced wheeze. When we repeated
216 the analysis based on children with asthma only, associations were weaker, probably
217 because the same characteristics that predicted persistence (exercise induced wheeze,
218 positive exercise test) had already been used by the clinicians to decide on a diagnosis of
219 asthma.

220 A few studies have examined the prediction of prognosis by clinical testing, but ours is the
221 only study to have done this for so many clinical tests in school-aged children referred to a
222 respiratory outpatient clinic. We did not find an association between FEV₁ or bronchial
223 provocation test by methacholine at baseline and wheeze seven years later; previous
224 studies have reported contradictory findings. Both the CAMP cohort of 909 children aged 5-
225 12 years with diagnosed asthma and another Dutch clinical cohort study of 5-14 year-old
226 children diagnosed with asthma found that asthma persistence at ages 15-20 and 32-42,
227 respectively, was associated with decreased FEV₁ at school-age^{8,9}. In contrast, the
228 population-based Tasmanian cohort did not find an association between FEV₁ at age 7 and
229 wheeze persistence at age 29-32¹⁵. The CAMP study also found that a lower methacholine
230 provocation concentration was associated with asthma persistence from age 5-12 until age
231 15-20⁹. In contrast, the population based Dunedin cohort of 613 children reporting wheeze at
232 age 9 and a Norwegian cohort of 62 children reporting asthma at age 10, found that

233 bronchial provocation test by methacholine was not associated with persistence at age 26
234 and 16 respectively, which is in line with our findings^{12,28}. The Norwegian cohort did not find
235 bronchial provocation test by exercise to be associated with asthma persistence from age 10
236 to age 16, which is in contrast to the association we found²⁸. This heterogeneity between
237 studies could be because children with wheeze from population-based cohorts might have
238 milder disease than those in clinical studies. FeNO was not associated with wheeze
239 persistence in children aged 6-16 years old suspected for asthma in our study. In contrast,
240 FeNO was reported to predict asthma in pre-school children with wheeze²⁹⁻³². To our
241 knowledge, no studies assess the predictive value of FeNO on wheeze persistence at school
242 age. Available publications assessed the predictive value of FeNO on asthma control,
243 relapse or exacerbations in asthmatic children, but with a short follow-up³³⁻³⁶. .
244 Our observation that frequent attacks of wheeze at school age predicted wheeze persistence
245 seven years later is in line with findings from the Melbourne and Tasmanian cohorts^{10,11}. In
246 contrast to their findings, we found no significant association between either eczema or hay
247 fever at baseline and wheeze persistence. This could be because those cohorts used
248 different outcomes—severe wheeze and atopic asthma, respectively—or simply because we
249 had low numbers and limited power.

250 A possible limitation of our study was that the bronchial provocation tests were done within a
251 short period of time. This could have influenced the methacholine test result, which was
252 performed after the exercise test on the same day and was positive in 76% of the children.
253 Most likely the bronchial provocation test by mannitol was not influenced by the short time
254 interval. We assured an appropriate interval of at least 24 hours without a change in
255 respiratory health or medication in this time interval. A second limitation was the small
256 sample size, which limited statistical power and did not allow us to perform a multivariable
257 analysis including all symptoms and test results simultaneously. Adolescents might have
258 underreported respiratory symptoms, which might have led to an underestimation of the
259 proportion of adolescents with wheeze. However, since this underreporting is not likely to be

260 associated with symptoms or positive test results at baseline, this should not have influenced
261 the results relating to risk factors.

262 The main strength of our study is its clinical design, which reflects the typical mix of patients
263 in a paediatric outpatient clinic. All children were first-time referrals to the paediatric
264 respiratory clinic for evaluation of possible asthma. Therefore, the study population is
265 representative of daily clinical work, in contrast to many clinical studies that selectively
266 include well-defined moderate to severe asthmatics and leave out patients with unclear
267 degrees of airway reactivity. Our study also profited from a very detailed baseline
268 examination. Children in the study had an extensive array of examinations for lung function,
269 BPT and allergy, which allowed us to assess the contribution of clinical tests in predicting
270 long-term wheeze in addition to reported symptoms among those referred for evaluation of
271 possible asthma.

272

273 **Conclusion**

274 This study is an initial step towards finding out whether clinical tests can predict wheeze later
275 in life. Though clinical tests might be important for asthma diagnosis, our results suggest that
276 they do not strongly predict prognosis of wheeze. In contrast, our data underline the
277 importance of a detailed history, as school-age children reporting exercise-related wheeze
278 and wheeze triggered by allergens were at higher risk and thus might profit from more
279 frequent follow-up.

280

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293

294 **Author contributions**

295 Claudia Kuehni and Jürg Barben conceptualised and designed the study. Daniel Trachsel
296 and Jürg Barben supervised data collection. Carmen de Jong analysed the data and drafted
297 the manuscript. Eva Pedersen and Myrona Goutaki supported the statistical analysis and
298 gave input for interpretation of the data. All authors critically revised the manuscript and
299 approved the final manuscript as submitted.

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302 References

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- 304 1. Braig S, Brandt S, Wabitsch M, Florath I, Brenner H, Rothenbacher D, Genuneit J. Age-specific
305 influence of wheezing phenotypes on pre-adolescent and adolescent health-related quality of
306 life. *Pediatr Allergy Immunol.* 2014;25(8):781-787.
- 307 2. Jurca M, Pescatore AM, Goutaki M, Spycher BD, Beardsmore CS, Kuehni CE. Age-related
308 changes in childhood wheezing characteristics: A whole population study. *Pediatr Pulmonol.*
309 2017;52(10):1250-1259.
- 310 3. Stevens CA, Turner D, Kuehni CE, Couriel JM, Silverman M. The economic impact of preschool
311 asthma and wheeze. *Eur Respir J.* 2003;21(6):1000-1006.
- 312 4. Fuchs O, Bahmer T, Rabe KF, von Mutius E. Asthma transition from childhood into adulthood.
313 *Lancet Respir Med.* 2017;5(3):224-234.
- 314 5. Spycher BD, Cochrane C, Granell R, Sterne JAC, Pedersen E, Gaillard EA, Henderson J, Kuehni
315 CE. Temporal stability of multiple trigger and episodic viral wheeze in early childhood. *Eur*
316 *Respir J.* 2017;50(5):1700014.
- 317 6. van Wonderen KE, Geskus RB, van Aalderen WM, Mohrs J, Bindels PJ, van der Mark LB, Ter
318 Riet G. Stability and predictiveness of multiple trigger and episodic viral wheeze in
319 preschoolers. *Clin Exp Allergy.* 2016;46(6):837-847.
- 320 7. Sears MR. Predicting asthma outcomes. *J Allergy Clin Immunol.* 2015;136(4):829-836.
- 321 8. Vonk JM, Postma DS, Boezen HM, Grol MH, Schouten JP, Koeter GH, Gerritsen J. Childhood
322 factors associated with asthma remission after 30 year follow up. *Thorax.* 2004;59(11):925-
323 929.
- 324 9. Covar RA, Strunk R, Zeiger RS, Wilson LA, Liu AH, Weiss S, Tonascia J, Spahn JD, Szeffler SJ.
325 Predictors of remitting, periodic, and persistent childhood asthma. *J Allergy Clin Immunol.*
326 2010;125(2):359-366.
- 327 10. Wolfe R, Carlin JB, Oswald H, Olinsky A, Phelan PD, Robertson CF. Association between allergy
328 and asthma from childhood to middle adulthood in an Australian cohort study. *Am J Respir*
329 *Crit Care Med.* 2000;162(6):2177-2181.
- 330 11. Martin PE, Matheson MC, Gurrin L, Burgess JA, Osborne N, Lowe AJ, Morrison S, Mészáros D,
331 Giles GG, Abramson MJ, et al. Childhood eczema and rhinitis predict atopic but not nonatopic
332 adult asthma: a prospective cohort study over 4 decades. *J Allergy Clin Immunol.*
333 2011;127(6):1473-1479.
- 334 12. Sears MR, Greene JM, Willan AR, Wiecek EM, Taylor DR, Flannery EM, Cowan JO, Herbison GP,
335 Silva PA, Poulton R. A longitudinal, population-based, cohort study of childhood asthma
336 followed to adulthood. *N Engl J Med.* 2003;349(15):1414-1422.
- 337 13. Andersson M, Hedman L, Bjerg A, Forsberg B, Lundback B, Ronmark E. Remission and
338 persistence of asthma followed from 7 to 19 years of age. *Pediatrics.* 2013;132(2):e435-442.
- 339 14. Burgess JA, Walters EH, Byrnes GB, Matheson MC, Jenkins MA, Wharton CL, Johns DP,
340 Abramson, Hopper JL, Dharmage SC. Childhood allergic rhinitis predicts asthma incidence and
341 persistence to middle age: a longitudinal study. *J Allergy Clin Immunol.* 2007;120(4):863-869.
- 342 15. Jenkins MA, Hopper JL, Bowes G, Carlin JB, Flander LB, Giles GG. Factors in childhood as
343 predictors of asthma in adult life. *BMJ.* 1994;309(6947):90-93.
- 344 16. Oswald H, Phelan PD, Lanigan A, Hibbert M, Bowes G, Olinsky A. Outcome of childhood asthma
345 in mid-adult life. *BMJ.* 1994;309(6947):95-96.
- 346 17. Barben J, Kuehni CE, Strippoli MP, Schiller B, Hammer J, Trachsel D, Swiss Paediatric
347 Respiratory Research Group. Mannitol dry powder challenge in comparison with exercise
348 testing in children. *Pediatr Pulmonol.* 2011;46:842-848.
- 349 18. Barben J, Strippoli MP, Trachsel D, Schiller B, Hammer J, Kuehni CE. Effect of Mannitol dry
350 powder challenge on exhaled nitric oxide in children. *PLoS One.* 2013;8(1):e54521.

- 351 19. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, Mitchell EA, Pearce N, Sibbald
352 B, Stewart AW, et al. International study of asthma and allergies in childhood (ISAAC):
353 rationale and methods. *Eur Respir J*. 1995;8:483-491.
- 354 20. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, MacIntyre NR, McKay RT,
355 Wanger JS, Anderson SD, et al. Guidelines for methacholine and exercise challenge testing-
356 1999. *Am J Respir Crit Care Med*. 2000;161(1):309-329.
- 357 21. Anderson SD, Brannan J, Spring J, Spalding N, Rodwell LT, Chan K, Gonda I, Walsh A, Clark AR.
358 A new method for bronchial-provocation testing in asthmatic subjects using a dry powder of
359 mannitol. *Am J Respir Crit Care Med*. 1997;156(3.1):758-765.
- 360 22. Standardization of Spirometry, 1994 Update. American Thoracic Society. *Am J Respir Crit Care*
361 *Med*. 1995;152(3):1107-1136.
- 362 23. Recommendations for standardized procedures for the on-line and off-line measurement of
363 exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children-1999. This
364 official statement of the American Thoracic Society was adopted by the ATS Board of
365 Directors, July 1999. *Am J Respir Crit Care Med*. 1999;160(6):2104-2117.
- 366 24. Barben J RJ. Measurement of bronchial responsiveness in children. In: Hammer J, Eber E,
367 editors. Paediatric pulmonary function testing. *Prog Respir Res*. Basel: Karger;2005;33:125-
368 136.
- 369 25. Barben J, Roberts M, Chew N, Carlin JB, Robertson CF. Repeatability of bronchial
370 responsiveness to mannitol dry powder in children with asthma. *Pediatr Pulmonol*.
371 2003;36(6):490-494.
- 372 26. Zapletal A, Paul T. Lung function in children and adolescents. Methods, reference values. *Prog*
373 *Respir Res*. 1987;22:1-220.
- 374 27. Braun-Fahrlander C, Wüthrich B, Gassner M, Gritze I, Neu U, Varonier H. Prävalenz und
375 Risikofaktoren einer allergischen Sensibilisierung bei Schulkindern in der Schweiz.
376 *Allergologie*. 1999;22:54-64.
- 377 28. Riiser A, Hovland V, Carlsen KH, Mowinckel P, Lodrup Carlsen KC. Does bronchial
378 hyperresponsiveness in childhood predict active asthma in adolescence? *Am J Respir Crit Care*
379 *Med*. 2012;186(6):493-500.
- 380 29. Pijnenburg MW. The Role of FeNO in Predicting Asthma. *Front Pediatr*. 2019;7:41.
- 381 30. Singer F, Luchsinger I, Inci D, Knauer N, Latzin P, Wildhaber JH, Moeller A. Exhaled nitric oxide
382 in symptomatic children at preschool age predicts later asthma. *Allergy*. 2013;68(4):531-538.
- 383 31. Elliott M, Heltshe SL, Stamey DC, Cochrane ES, Redding GJ, Debley JS. Exhaled nitric oxide
384 predicts persistence of wheezing, exacerbations, and decline in lung function in wheezy
385 infants and toddlers. *Clin Exp Allergy*. 2013;43(12):1351-1361.
- 386 32. Caudri D, Wijga AH, Hoekstra MO, Kerkhof M, Koppelman GH, Brunekreef B, Smit HA, de
387 Jongste JC. Prediction of asthma in symptomatic preschool children using exhaled nitric oxide,
388 Rint and specific IgE. *Thorax*. 2010;65(9):801-807.
- 389 33. Lehtimäki L, Csonka P, Makinen E, Isojarvi J, Hovi SL, Ahovuo-Saloranta A. Predictive value of
390 exhaled nitric oxide in the management of asthma: a systematic review. *Eur Respir J*.
391 2016;48(3):706-714.
- 392 34. Pijnenburg MW, Hofhuis W, Hop WC, De Jongste JC. Exhaled nitric oxide predicts asthma
393 relapse in children with clinical asthma remission. *Thorax*. 2005;60(3):215-218.
- 394 35. Visitsunthorn N, Mahawichit N, Maneechotesuwan K. Association between levels of fractional
395 exhaled nitric oxide and asthma exacerbations in Thai children. *Respirology*. 2017;22(1):71-
396 77.
- 397 36. Yang S, Park J, Lee YK, Kim H, Hahn YS. Association of longitudinal fractional exhaled nitric
398 oxide measurements with asthma control in atopic children. *Respir Med*. 2015;109(5):572-
399 579.

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402 **Supplementary data**

403 **E-table 1** Comparison of characteristics of the children included in the follow-up study and
404 the children that did not take part in the follow-up study

405

406 **E-table 2** Association between reported exercise-induced wheeze and exercise test result at
407 baseline N=76

408

409 **E-table 3** Association between baseline factors and wheeze at follow-up in children with
410 diagnosed asthma

411

412 **E-table 4** Association between baseline factors and frequent wheeze at follow up

413

414 **E-figure 1** Flowchart of patient recruitment at baseline and follow-up

415

416 **E-text:** methods of clinical tests

417 **Supplementary questionnaire 1:** Baseline questionnaire in German (original)

418 **Supplementary questionnaire 2:** Baseline questionnaire in English

419 **Supplementary questionnaire 3:** Follow-up questionnaire in German (original)

420 **Supplementary questionnaire 4:** Follow-up questionnaire in English