

ORIGINAL ARTICLE

A longitudinal assessment of circulating YKL-40 levels in preschool children with wheeze

Anna James^{1,a}, Katarina Stenberg Hammar^{2,3,a}, Lovisa Reinius⁴, Jon R. Konradsen^{2,3,5}, Sven-Erik Dahlén¹, Cilla Söderhäll^{3,4} & Gunilla Hedlin^{2,3}

¹Experimental Asthma and Allergy Research, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; ²Astrid Lindgren Children's Hospital, Karolinska University Hospital, Stockholm, Sweden; Departments of ³Women's and Children's Health; ⁴Biosciences and Nutrition; ⁵Department of Medicine, Immunology and Allergy Unit, Karolinska Institutet, Stockholm, Sweden

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Keywords

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Correspondence

Anna James, Experimental Asthma and Allergy Research, Institute of Environmental Medicine, Karolinska Institutet, PO Box 287, Stockholm SE-171 77, Sweden
E-mail: anna.james@ki.se

All authors are associated with the Centre for Allergy Research, Karolinska Institutet, Stockholm, Sweden.

^aShared first authorship.

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Abstract

Background: The chitinase-like protein YKL-40 (*CHI3L1*) is elevated in the circulation of adults and schoolchildren with chronic severe asthma. It is unknown whether YKL-40 is altered in younger, preschool children with wheeze, acute or chronic. We therefore examined YKL-40 in preschool children during an acute episode of wheeze and during remission, in comparison with healthy controls.

Methods: Blood was obtained from 128 children (aged 6–44 months) at the emergency department during an acute episode of wheeze, and at two follow-up visits (approximately 3 months and 1 year later), as well as from 100 age-matched healthy controls on one occasion. Plasma YKL-40 levels were examined in relation to *CHI3L1* rs4950928 genotype and clinical characteristics including Asthma Predictive Index, medication use, time spent with respiratory symptoms, atopic status, and blood leukocytes.

Results: Children with wheeze had higher median YKL-40 levels at the acute visit (14.7 (11.5–22.6) ng/ml, $p < 0.001$) and 3-month follow-up (15.9 (11.5–20.2), $p < 0.001$) compared to the 1-year follow-up (11.9 (9.5–17.3)). YKL-40 levels in healthy controls (13.6 (11.0–17.0)) tended to be lower than those during acute wheeze ($p = 0.07$) and 3-month follow-up ($p = 0.04$), but were no different at the 1-year follow-up. *CHI3L1* rs4950928 affected YKL-40 in all subjects, with highest levels present in those with the CC genotype ($p < 0.001$). Genotype frequency was similar in the two subject groups. YKL-40 levels showed a positive correlation with blood neutrophil counts but no consistent relationships with clinical characteristics of relevance to continuous wheeze.

Conclusion: YKL-40 levels were elevated during acute wheeze in preschool children, a finding which may be related to current neutrophilic inflammation, but YKL-40 was not associated with characteristics of persistent wheeze in this young cohort.

Easily attainable biomarkers, able to reflect chronic inflammation, and remodeling processes within the airways are lacking, but one protein that has shown potential for this purpose is the chitinase-like protein YKL-40, which is elevated in the blood of both school-age children and adults with severe asthma (1, 2). YKL-40 is suggested to be involved in airway remodeling due to its effect on smooth muscle proliferation (3) and correlations with subepithelial fibrosis and bronchial wall thickness (1, 2).

It is not known whether YKL-40 is involved in airway inflammation, either acute or more chronic, in very young

children with airway disease, although it is now well established that the development of asthma often begins in early childhood (4). The biological mechanisms underlying preschool wheeze remain poorly understood, although there is recent evidence showing that children with rhinovirus-induced wheeze have altered exhaled biomarkers both during symptoms and after resolution, possibly reflecting the altered molecular profile which underlies an increased risk of developing asthma (5). Furthermore, even very young children with symptoms of wheeze may display signs of airway remodeling such as a

thickened basement membrane and increased airway smooth muscle mass (6).

It was therefore our aim to examine YKL-40 levels in this longitudinal study of preschool children with wheeze, to address whether associations between YKL-40 and airway disease exist even in the very young. Children attending the emergency ward with acute, mostly virus-induced, wheeze were re-investigated at two follow-up visits. For comparison, YKL-40 levels were also examined in a reference group of age-matched healthy controls. Relationships between YKL-40 and clinical factors of possible importance to the later development of asthma were examined, as well as potential effects of a common polymorphism in the *CHI3L1* gene, rs4950928 (-131C>G), which has been suggested to be associated with features of asthma and increased serum YKL-40 (7).

Methods

Study population and data collection

Children aged 6–44 months ($n = 156$) were enrolled at the emergency ward at Astrid Lindgren's Children's Hospital, Karolinska University Hospital, Stockholm, Sweden, when seeking care for acute episodes of wheeze. Exclusion criteria were prematurity (birth before 36 gestational weeks), other chronic diseases, or any simultaneous complications such as sepsis, bacterial pneumonia, or diabetes at the time point of

inclusion. Enrollment took place from October 2008 until June 2012. Blood samples were drawn at the emergency visit and at later remission visits to the study physician after approximately three and twelve months. Plasma samples for YKL-40 measurement were available from a subset of these children ($n = 128$ at acute visit, $n = 120$ at 3-month follow-up, and $n = 95$ at 1-year follow-up). Nasal swab tests for virus detection were obtained from children at the acute visit, and then again at the 3-month follow-up visit. Guardians were interviewed and filled out standardized questionnaires concerning disease symptoms, medication, hereditary, and environmental factors. Clinical data was available from 130 children that came back after the acute visit for follow-up and completed the interview and questionnaire. A positive Asthma Predictive Index was evaluated as described (8).

During the same time period, age-matched healthy controls ($n = 101$, of which $n = 100$ were available for YKL-40 analysis) were also recruited at the Children's Surgical Daycare Ward, Astrid Lindgren's Children's Hospital. Exclusion criteria for the controls were prematurity, a history of bronchial obstruction/asthma, or known sensitization to airborne allergens. A comparison of clinical data between these groups has been described previously (9). Blood samples were drawn at the same time as the insertion of an intravenous line prior to minor surgery, and the legal guardian filled out the same standardized questionnaires as the cases. The demographics of the children with wheeze and healthy controls are shown in Table 1.

Table 1 Basic characteristics of children with acute wheeze and healthy controls

	Acute wheeze ($n = 130$)	Healthy controls ($n = 101$)	p-value
Male (%)	65	79	0.02*
Age in months (median (min–max))			
acute visit	18 (6–42)	18 (6–44)	0.91†
3-month follow-up ($n = 130$)	20 (8–46)		
1-year follow-up ($n = 120$)	30 (18–57)		
Mother caucasian (%)	82	77	0.32*
Father caucasian (%)	84	76	0.15*
Parental asthma (%)	32	15	<0.01*
Current smoking at home (%)	20	19	0.92*
Eczema in child (%)	19	7	0.01*
Reported food sensitivity (%)	14	3	<0.01*
>6 respiratory infections a year (%)	66	19	<0.01*
Total IgE ≥ 100 kU/l (%(n))	19 (94)	13 (76)	0.30*
Total IgE, kU/l (median (min–max))	26.6 (0.7–810.7)	13.5 (0.2–414.1)	0.03†
Neutrophils, $10^9/l$ (median (min–max))‡			
acute visit	7.1 (1.6–27.8)	2.6 (0.9–11.7)	<0.001†
3-month follow-up	3.4 (0.5–12.9)		0.30†
1-year follow-up	3.5 (0.1–9.4)		0.22†
Eosinophils, $10^9/l$ (median (min–max))‡			
acute visit	0.1 (0.0–4.6)	0.2 (0.1–2.1)	<0.001†
3-month follow-up	0.3 (0.1–6.8)		0.001†
1-year follow-up	0.3 (0.1–1.1)		0.06†

*Chi-squared test.

†Mann–Whitney test.

‡Blood cell counts were analyzed in $n = 96$ control children, and in children with acute wheeze; $n = 149$ at visit 1, $n = 120$ at visit 2 and $n = 106$ at visit 3.

Laboratory analyses

Nasal swab tests for virus detection (Sigma-Virocult, Medical Wire & Equipment Co Ltd, Wiltshire, UK) were analyzed using a 15-virus RT-PCR assay (10), according to standard procedures at the Department of Clinical Microbiology, Karolinska University Hospital.

Blood samples were analyzed for total blood cell counts at the Karolinska University Hospital Laboratory at all visits. Total IgE was analyzed at the first revisit after 3 months. Plasma YKL-40 levels were measured in samples collected at the three visits, by ELISA according to the manufacturer's instructions (Human Chitinase 3-like 1 DuoSet ELISA Development Kit, R&D Systems, Abingdon, United Kingdom). In short, two different dilutions were made for each sample from which an average was obtained, and all samples were analyzed in duplicate, in random order, and presented as ng/ml. The detection limit of this assay was 31 pg/ml, interassay variability was 3%, and intra-assay variability was 14%.

Genotyping

DNA was available from 123 children with acute wheeze and 96 controls. The -131 C>G polymorphism in the *CH3L1* gene (rs4950928) was analyzed using TaqMan allelic discrimination on the ABI Prism 7500 detection system according to the manufacturer's protocol (C_27832042_10, Applied Biosystems, Waltham, Massachusetts, USA). Reruns of 10% of the samples showed 100% concordance.

Ethics

The study protocol was approved by the Regional Ethics Committee of Stockholm, Karolinska Institutet (2008/378-31/4, 2014/399-31/3), and written informed consent was obtained from the legal guardians.

Statistical analyses

Basic comparisons were performed using GraphPad Prism statistical software (La Jolla, CA, USA), and a p -value <0.05 was accepted as significant. Normality was tested using the Kolmogorov–Smirnov test which showed that YKL-40 levels were skewed. Therefore, YKL-40 results are presented as median with interquartile range, and nonparametric tests were performed: the Wilcoxon signed-rank test for paired datasets, the Mann–Whitney test for unpaired datasets, and the Spearman rank test for univariate correlations. Regression analyses were performed using SPSS software following log transformation of YKL-40 levels (version 22, IBM Corporation, Armonk, NY, USA).

Results

Basic characteristics of children with wheeze and healthy controls

Comparing the characteristics of the children with acute wheeze and the healthy controls revealed that the children

with acute wheeze were more likely to have parental asthma, reported food sensitivity, and eczema, as well as a higher frequency of airway infections over a 1-year period (Table 1). The number of children with total IgE levels >100 kUA/l was not significantly different between the two groups, but absolute total IgE levels were higher in children with wheeze ($p = 0.03$). In children with acute wheeze, blood neutrophils were higher than in healthy controls ($p < 0.001$), whereas blood eosinophil numbers were lower ($p < 0.001$).

YKL-40 levels in children with wheeze and healthy controls

YKL-40 levels were examined in patients with wheeze at the acute visit, the 3-month follow-up, and the 1-year follow-up. Paired comparisons in patients between these visits revealed that YKL-40 levels at the 1-year follow-up (11.9 (9.5–17.3) ng/ml) were lower than at both the acute visit (14.7 (11.5–22.6), $p < 0.001$) and the 3-month follow-up (15.9 (11.5–20.2), $p < 0.001$, Fig. 1a). Levels were no different between the acute visit and the 3-month follow-up ($p = 0.7$). YKL-40 levels in healthy controls (13.6 (11.0–17.0)) tended to be lower than those measured during acute wheeze ($p = 0.07$) and were significantly lower than at the 3-month follow-up (0.04), but were similar to YKL-40 levels at the 1-year follow-up ($p = 0.2$). Children experiencing wheeze at either of the follow-up visits were removed from this analysis.

The *CH3L1* rs4950928 genotype strongly affected circulating YKL-40 levels in all subjects with the highest levels observed in those with the CC genotype and lowest levels in those with GG ($p < 0.001$, Kruskal–Wallis three-way comparison, all subjects). When stratifying patients according to rs4950928 genotype, YKL-40 levels became significantly greater than those in healthy controls (15.2 (12.7–17.9) ng/ml) at both the acute visit (17.4 (13.3–23.8), $p = 0.05$) and the 3-month follow-up (16.9 (13.6–22.4), $p = 0.05$) in the CC group (Fig. 1b). For CC patients, levels were also lower at the 1-year follow-up (14.9 (11.4–17.4), $p = 0.01$) than the two previous visits but were no different to healthy controls. Fewer significant differences emerged for the less common CG and GG genotypes although the numbers of patients in these subgroups was also lower. As shown in Fig. 1c, there was no difference in the distribution of the CC, CG, and GG *CH3L1* rs4950928 genotypes between healthy controls (CC 55%, CG 39%, GG 6%) and children with wheeze (CC 63%, CG 31%, GG 6%, chi-squared test, $p = 0.51$).

Relationships between YKL-40 and clinical characteristics

To examine whether YKL-40 levels showed any difference between children with more severe compared to milder symptoms, the children with wheeze were divided into those who reported symptoms of wheeze for more or less than 25% of the time over the past year at the 1-year follow-up visit, those with or without a positive Asthma Predictive Index, and those who were receiving continuous therapy with inhaled corticosteroids or montelukast, at the three visits (Fig. 2). Overall, there were few differences in YKL-40 levels between these subgroups of children, other than lower levels of YKL-40

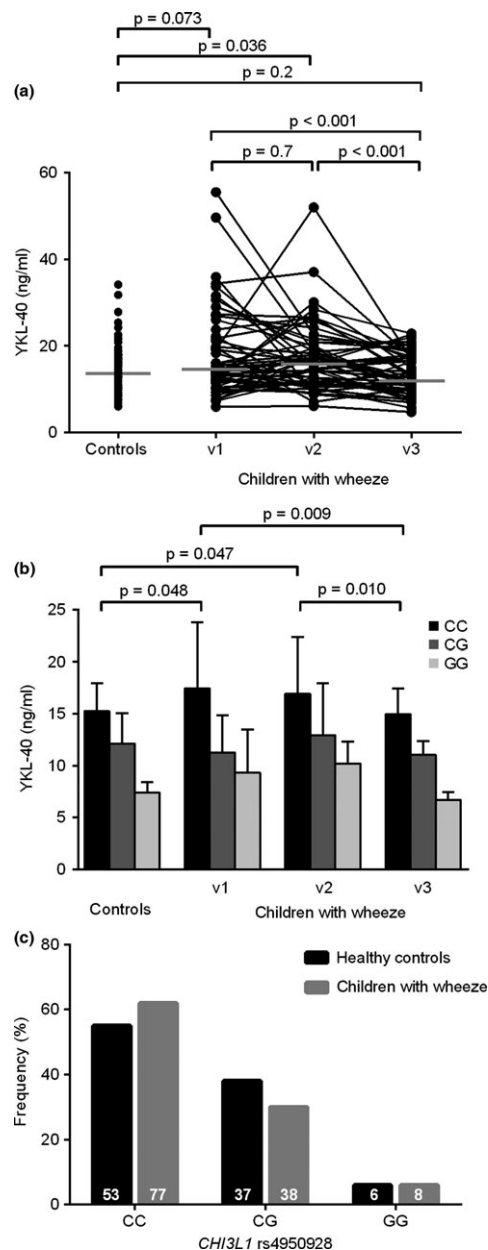


Figure 1 a) YKL-40 levels are shown in children with wheeze at the acute visit (v1), the 3-month follow-up (v2), and at the 1-year follow-up visit (v3), as well as in healthy controls (n = 100). Children with missing YKL-40 data at any of these three visits were excluded, as were those experiencing wheeze at any of the follow-up visits resulting in n = 54. Results are presented as individual data points with median bars. b) YKL-40 levels are shown in relation to *CH13L1* rs4950928 genotype for healthy controls (n = 94) and children with acute wheeze at the three different visits (v1 n = 95, v2 n = 98, v3 n = 83). Again, children experiencing wheeze at any of the follow-up visits were excluded. Results are presented as median with interquartile range. c) Distribution of *CH13L1* rs4950928 genotypes among healthy controls (n = 96) and children with acute wheeze (n = 123), the actual numbers of children with each genotype are shown in the columns.

in those who wheezed for less than 25% of the time at the 1-year follow-up visit, and higher levels of YKL-40 in those who were receiving continuous treatment with montelukast at the acute visit.

Correlations between YKL-40 and relevant continuous variables were examined both in healthy controls and children with acute wheeze (Table 2). Significant correlations were found between YKL-40 levels and neutrophil numbers in all subjects and remained after adjustment for *CH13L1* rs4950928. Neutrophil numbers were significantly higher in patients with acute wheeze compared to controls (Table 1) and followed a similar pattern to YKL-40 levels at the different visits, being lower at the 1-year follow-up than at the acute visit (Fig. S1). No significant correlations were observed between YKL-40 with total IgE or eosinophil numbers. A significant inverse relationship between YKL-40 levels and age was observed in healthy controls, but not in children with acute wheeze.

Multiple regression analyses were performed to examine factors significantly affecting YKL-40 levels in children with acute wheeze, when adjusting for relevant variables (Table 3). YKL-40 levels remained significantly associated with genotype, blood neutrophil numbers, and continuous medication with montelukast.

Possible relationships between YKL-40 and clinical characteristics were examined in patients grouped according to *CH13L1* rs4950928 genotype, either CC or CG and GG, but no differences were observed (Table S1).

YKL-40 levels in relation to virus infections

Of the children attending the emergency department with acute wheeze, testing for a panel of 15 different virus strains revealed that virus was detected in 86 of 126 children (68%), (most commonly RV in 40%, followed by RSV in 17%) (Table S2). No significant difference in YKL-40 levels was observed in children with acute wheeze when comparing all those with and without detectable virus infection (Fig. S2).

Discussion

In this longitudinal study of preschool children with wheeze, we explored associations between airway disease and levels of YKL-40, measured at an acute episode of wheeze and two follow-up visits during remission. We show that acute wheeze in children aged between 6 months and 4 years is associated with increased circulating YKL-40 compared to both healthy controls and levels at the 1-year follow-up visit, following stratification for *CH13L1* rs4950928 CC genotype. This single nucleotide polymorphism strongly affects YKL-40 levels whereby higher levels are found in those with the more common CC genotype, compared to CG and GG. Although YKL-40 levels were increased during the acute episode of airway inflammation, they were not different between the wheeze group and healthy controls at the 1-year follow-up visit and were not increased in children with more persistent wheeze at this time point. YKL-40 levels showed a positive correlation

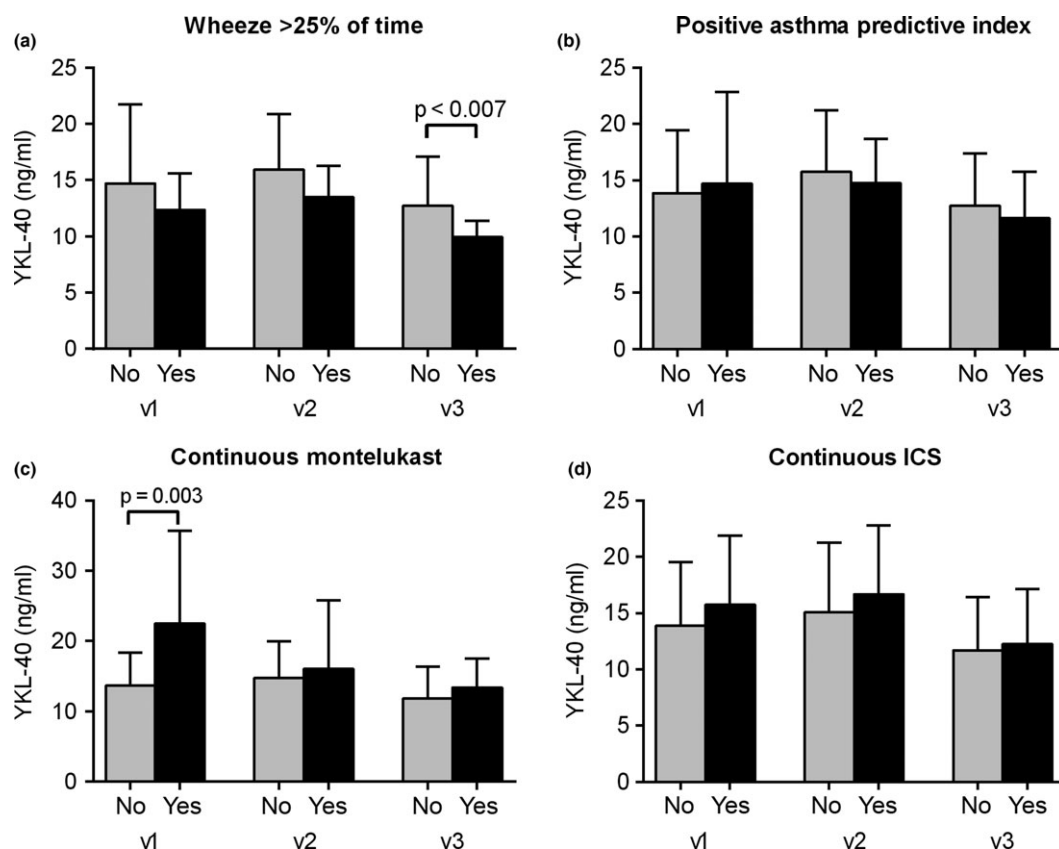


Figure 2 YKL-40 levels in children at the three visits grouped according to possible indicators of disease severity. a) Children who reported symptoms of wheeze for more than 25% of the time at the 1-year follow-up (no $n = 98$, yes $n = 18$). b) Children with a positive Asthma Predictive Index (no $n = 64$, yes $n = 55$). c) Children who were on continuous treatment with montelukast (no $n = 83$ – 109 , yes $n = 12$ – 22 , at the different visits). d) Children on continuous treatment with inhaled corticosteroids (ICS) (no $n = 63$ – 100 , yes $n = 21$ – 52 , at the different visits). Results are shown for children where both YKL-40 measurements and clinical information regarding symptoms and medication were available. The three different visits are v1 (acute visit), v2 (3-month follow-up), and v3 (1-year follow-up). Data are presented as median with interquartile range.

Table 2 Correlations between YKL-40 levels and continuous variables

	Healthy controls			Acute wheeze		
	Spearman Rank Correlation Coefficient	p-value	p-value (linear regression with adjustment for rs4950928 genotype)	Spearman Rank Correlation Coefficient	p-value	p-value (linear regression with adjustment for rs4950928 genotype)
Neutrophils, $10^9/l$	0.24	0.02	<0.01	0.29	<0.01	0.02
Eosinophils, $10^9/l$	0.12	0.28	0.32	−0.13	0.49	0.35
Total IgE, kU/l	−0.19	0.11	0.10	0.05	0.69	0.81
Age, months	−0.24	0.02	<0.01	0.14	0.15	0.36

with blood neutrophil numbers, which were also elevated during the episode of acute wheeze.

To our knowledge, it has not previously been examined whether circulating YKL-40 is affected by an acute episode of severe wheeze requiring emergency care in young children. Here,

we find elevated YKL-40 at the emergency visit compared to levels in the same patients 1 year later. In adults, allergen challenge may cause local increases in YKL-40 as revealed by increased sputum and BAL levels (11–13), although disease exacerbations of unknown cause (not requiring hospitalization)

Table 3 Multiple regression model showing variables affecting YKL-40 levels at the acute visit in children with wheeze

	p-value	95% CI	
		Lower bound	Upper bound
Montelukast continuous	<0.01	0.32	1.1
Neutrophils	0.04	0.001	0.04
YKL-40 Genotype CC	<0.01	0.26	0.63

The variables included in the model were age, gender, family history of asthma, continuous inhaled corticosteroid therapy, continuous montelukast therapy, blood neutrophil number, and *CHI3L1* rs4950928 genotype. Variables with significant associations are shown above.

had no effect on serum YKL-40 compared to baseline (11). However, in adults hospitalized with severe pneumonia due to *Streptococcus pneumoniae* infection, YKL-40 levels in the circulation were almost ninefold higher than in controls (14), suggesting that a detectable increase in YKL-40 in the circulation could reflect a more invasive process of inflammation. Although we were not able to directly relate the increase in YKL-40 to the underlying cause of the acute wheeze, it could be associated with neutrophilic inflammation. In the present study, there was a moderate, yet significant, positive correlation between YKL-40 and neutrophil numbers which were also elevated at the acute visit. Neutrophils are a known source of YKL-40 and are increased in the circulation during acute infections in adults (14, 15). In line with the current findings, preschool children with acute viral wheeze have been found to have both increased neutrophil numbers and markers of neutrophil activation (16), possibly prior to recruitment to the airways. As the children do not have chronic inflammation, the levels of neutrophils and also levels of YKL-40 may be normalized when the symptoms of the exacerbation have passed.

Although the underlying biological pathways were not examined, one may speculate that the increase in YKL-40 arises from the activation of neutrophils as mentioned, but also from macrophages and epithelial cells, known cellular sources of YKL-40 (17). Rhinovirus infection disturbs the tight junctions between epithelial cells and induces a strong cytokine-driven innate immune inflammation (18). This process may activate tissue macrophages by epithelial interferon release, thereby further contributing to the local production and release of YKL-40 (19). The biological processes that YKL-40 itself is involved in, which are of relevance to airway disease, may include its effects on tissue remodeling. YKL-40 has been shown to induce smooth muscle and fibroblast proliferation (3, 20), promote angiogenesis (21), and modulate collagen fibril formation (22).

CHI3L1 rs4950928 genotype was found to have a strong effect on circulating YKL-40 levels confirming several previous observations (1, 7, 11). It is not entirely clear whether this polymorphism is associated with features of asthma or airway disease, as different studies of different populations have shown conflicting findings (7, 23–25). Here, a difference between the children with acute wheeze and healthy controls

became apparent following stratification for rs4950928 CC genotype, those in whom circulating YKL-40 levels are highest, although there was no significant difference in the distribution of the rs4950928 CC, CG, and GG genotypes among healthy controls, compared to children with acute wheeze. Also, no differences in clinical characteristics were observed between the CC, CG, and GG groups.

The chitinase-like protein YKL-40 has previously been found to be increased in both schoolchildren and adults with severe, chronic asthma, leading to the proposal that it may be a potential biomarker capable of reflecting airway inflammation (1, 2, 11). It is not clear whether this is also the case in very young children with persistent wheeze. At the 1-year follow-up visit, YKL-40 levels in the wheeze group as a whole were no different to those in healthy controls. Dividing children into those with more persistent symptoms of wheeze, a positive Asthma Predictive Index, or those on continuous asthma treatment as possible indicators of more continuous airway disease was generally not associated with higher YKL-40 levels. Interestingly, significantly higher YKL-40 levels were observed in children taking montelukast at the acute visit, which could indirectly suggest higher YKL-40 levels in a group of children with more severe airway disease requiring asthma medication although this difference was not observed at the follow-up visits during remission. Taken together, these findings suggest that YKL-40 is not a clinically useful biomarker of persistent airway disease in the very young, possibly because the biological processes in which YKL-40 is involved in adult asthma are not occurring at this early age. Later follow-up of the same group of children will enable investigation of this possibility. In line with our observations, Ober et al. (7) found that serum YKL-40 levels at birth, 1, 3, and 5 years of age were not significant predictors of a later asthma diagnosis at 6 years of age, and similarly, Usemann et al. (26) found no significant association between cord blood YKL-40 levels and asthma in early childhood.

In the current study, we did not observe a clear relationship between YKL-40 levels and age. In adults, YKL-40 levels increase with age, regardless of the presence of inflammatory disease (27). In young children, YKL-40 levels have been shown to be higher at birth and then plateau up to 5 years of age (7). In healthy controls, a negative correlation with age was observed which could relate to higher levels at birth becoming lower with age; however, no such correlation was observed in the children with wheeze.

In conclusion, we show for the first time that in a study of preschool children aged 6 months to 4 years, circulating YKL-40 levels were increased during an acute episode of wheeze, a finding that could be related to current neutrophilic inflammation. We also provide reference values of YKL-40 levels in a large group of 100 age-matched healthy controls. However, circulating YKL-40 levels were not able to differentiate between children with symptoms of more continuous wheeze, possibly those who are likely to go on to develop chronic asthma, as compared to those with less symptoms and compared to the healthy children. YKL-40 is therefore unlikely to be a clinically useful biomarker of persistent wheeze in this very young age group.

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Supporting Information

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

Figure S1. Blood neutrophil counts in children with wheeze at the acute visit, the 3-month follow-up and the 1-year follow-up visit. Children with missing YKL-40 data at any of these three visits were excluded, as were those experiencing wheeze at any of the follow-up visits resulting in $n = 67$. Neutrophils numbers were significantly higher at the acute visit than at the 3-month and 1-year follow-up visits. Results show individual data points.

Figure S2. YKL-40 levels in children with acute wheeze in relation to the presence of detectable viral infection. In the group as a whole there was no

significant difference in YKL-40 level between those with and without detectable virus, $p = 0.071$. Stratification for genotype did not reveal significant differences in YKL-40 levels when comparing those with and without detectable virus and CC genotype only. In patients with CG or GG genotype, YKL-40 levels were lower in those with detectable virus, $p = 0.002$. Results show individual data points and median bars.

Table S1. Clinical characteristics, history of respiratory infections and medication at the 3-month follow-up in patients with wheeze, grouped according to CHI3L1 rs4950928 genotype, either CC or CG/GG.

Table S2. Virus detection in children with acute wheeze.