The chitinase-like protein YKL-40: A possible biomarker of inflammation and airway remodeling in severe pediatric asthma

Jon R. Konradsen, MD, PhD,[a,b,c]* Anna James, PhD,[c,d]* Björn Nordlund, RN,[a,b,c] Lovisa E. Reinius, PhD,[c,e] Cilla Söderhäll, PhD,[e] Erik Melén, MD, PhD,[d,f] Åsa Wheelock, PhD,[g] Karin C. Lödrup Carlsen, MD, PhD,[h] Marika Lidegran, MD, PhD,[a] Marri Verhoek,[i] Rolf G. Boot, PhD,[i] Barbro Dahlén, MD, PhD,[c,j] Sven Erik Dahlén, MD, PhD,[c,d] and Gunilla Hedlin, MD, PhD[a,b,c] *Stockholm and Huddinge, Sweden, Oslo, Norway, and Amsterdam, The Netherlands*

Background: Problematic severe childhood asthma includes a subgroup of patients who are resistant to therapy. The specific mechanisms involved are unknown, and novel biomarkers are required to facilitate treatment and diagnosis of therapy-resistant asthma. The chitinase-like protein YKL-40 has been related to asthma and airway remodeling.

Objectives: To compare serum YKL-40 levels in children with severe, therapy-resistant asthma (n = 34), children with controlled persistent asthma (n = 39), and healthy controls (n = 27), and to investigate correlations with biomarkers of inflammation and airway remodeling.

Methods: The study protocol included questionnaires, measurement of exhaled nitric oxide in exhaled air, blood sampling for inflammatory biomarkers, and high-resolution computed tomography of the lungs to identify bronchial wall thickening (therapy-resistant only). Serum YKL-40 levels were measured by ELISA, and all asthmatic children were genotyped for a *CHI3L1* promoter single nucleotide polymorphism (rs4950928).

Results: Serum YKL-40 levels were significantly higher in children with therapy-resistant asthma than in healthy children (19.2 ng/mL vs 13.8 ng/mL, $P = .03$). Among children with severe, therapy-resistant asthma, YKL-40 levels correlated with fraction of exhaled nitric oxide in exhaled air ($r = 0.48$, $P = .004$), blood neutrophils ($r = 0.63$, $P < .001$), and bronchial wall thickening on high-resolution computed tomography ($r = 0.45$, $P = .01$). Following adjustment for *CHI3L1* genotype, significantly greater levels of YKL-40 were found in children with therapy-resistant asthma than in children with controlled asthma.

Conclusions: YKL-40 levels are increased in children with severe, therapy-resistant asthma compared to healthy children, and also compared to children with controlled asthma following correction for genotype. (J Allergy Clin Immunol 2013;132:328-35.)

Key words: *Asthma, children, inflammation, airway remodeling, chitinases, imaging, genes*

A small proportion of children with asthma suffer from chronic symptoms and severe exacerbations despite treatment with high doses of currently available asthma medications.[1,2] The term problematic severe asthma has been proposed to classify these children of whom a subgroup suffers from severe, therapy-resistant asthma that does not respond satisfactorily to asthma therapy and probably requires novel therapeutic approaches.[3] There is an urgent need to find new and specific biomarkers to improve the diagnosis and treatment of patients with severe, therapy-resistant childhood asthma.[4,5]

A group of proteins recently discovered to be potential biomarkers of asthma are the chitinases and chitinase-like proteins. Chitin is a tough structural polysaccharide, the second most abundant biopolymer in nature after cellulose, used by a variety of organisms including insects, crustaceans, parasites, fungi, and bacteria to protect against harsh environmental conditions.[6] Although chitin is not present in the human body, we express enzymes capable of its degradation. Two members of this family, the enzymatically active chitotriosidase and the enzymatically inactive chitinase-like protein YKL-40, have been shown to be increased in the serum of asthmatic patients,[7,8] and levels of

From [a]Astrid Lindgren Children's Hospital, Karolinska University Hospital, [b]the Department of Women's and Children's Health, [c]the Centre for Allergy Research, [d]The National Institute of Environmental Medicine, and [e]the Department of Bioscience and Nutrition, Karolinska Institutet, [f]Sachs' Children and Youth Hospital, and [g]the Respiratory Medicine Unit, Department of Medicine, Karolinska Institutet, Stockholm; [h]the Department of Pediatrics, Oslo University Hospital and the University of Oslo, Oslo; [i]the Department of Medical Biochemistry, Academic Medical Center, University of Amsterdam, Amsterdam; and [j]the Division of Respiratory Medicine and Allergy Research, Department of Medicine, Karolinska University Hospital, Huddinge.

*These authors contributed equally to this work.

This study was supported by the Freemason Child House Foundation in Stockholm, the Konsul Th. C. Bergh's Foundation, the Swedish Asthma and Allergy Association's Research Foundation, the Centre for Allergy Research at Karolinska Institutet, the Pediatric Research Foundation of Astrid Lindgren Children's Hospital, the Swedish Heart-Lung Foundation, Karolinska Institutet, the Bernard Osher Initiative for Research on Severe Asthma, Stockholm County Council Research Funds, and VINNOVA, Swedish Governmental Agency for Innovation Systems.

Disclosure of potential conflict of interest: B. Dahlén is a Board member for Actelion DMC; has received one or more grants from or has one or more grants pending with the Swedish Heart-Lung Foundation, the Swedish MRC, and the Swedish Asthma and Allergy Foundation; and has received one or more payments for lecturing from or is on the speakers' bureau for Novartis and for Meda. S. E. Dahlén has been supported by one or more grants from the Swedish MRC, the Swedish Heart-Lung Foundation, and the Stockholm County Council Research Funds; has consultancy arrangements with GSK & AZ Respiratory; and has received one or more payments for lecturing from or is on the speakers' bureau for Nigaard Pharmaceuticals. J. R. Konradsen has received grants from the Freemason Child House Foundation in Stockholm, the Konsul Th. C. Bergh's Foundation, the Swedish Asthma and Allergy Association's Research Foundation, the Centre for Allergy Research at Karolinska Institutet, the Pediatric Research Foundation of Astrid Lindgren Children's Hospital, and the Swedish Heart-Lung Foundation; and has received payment for lecturing from Novartis and Thermo Fisher Scientific. K. C. Lödrup Carlsen has received one or more public grants, has received or has one or more grants pending with the Kloster Foundation, and has received one or more payments for lecturing from or is on the speakers' bureau for GSK. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication September 27, 2012; revised March 6, 2013; accepted for publication March 8, 2013.

Available online April 26, 2013.

Corresponding author: Jon R. Konradsen, MD, PhD, Astrid Lindgren Children's Hospital, H1:02, Karolinska University Hospital, SE-17176 Stockholm, Sweden. E-mail: jon.konradsen@karolinska.se.

0091-6749/$36.00

© 2013 American Academy of Allergy, Asthma & Immunology

http://dx.doi.org/10.1016/j.jaci.2013.03.003

Abbreviations used
FENO: Fraction of exhaled nitric oxide in exhaled air
HRCT: High-resolution computerized tomography

YKL-40 have also been shown to correlate with markers of disease severity.[8] Interestingly, a promoter single nucleotide polymorphism in the gene encoding YKL-40, *CHI3L1* rs4950928 (-131C/G) has also been associated with features of asthma and increased circulating YKL-40 levels in serum.[9] Chitotriosidase activity is also subject to strong genetic regulation in that individuals homozygous for a 24-bp duplication in exon 10 of *CHIT1*, the gene coding for chitotriosidase, display complete enzyme inactivity.[10] However, little is known about the expression and regulation of YKL-40 and chitotriosidase in relation to asthma in children.

Specifically, it has been suggested that the chitinase family members may be involved in the development of fibrosis and remodeling of the airway.[11] Bronchial wall thickening, identified by high-resolution computerized tomography (HRCT), can be assessed quantitatively on the basis of the number of visible bronchi at 3 different levels of the lung. Increased bronchial wall thickening has been shown to be a consistent finding in children with severe asthma compared with healthy controls.[12] The thickness of the bronchial wall has been shown to correlate with the thickness of the reticular basement membrane and the airway epithelium,[13-15] supporting the assessment of bronchial wall thickening as an appropriate marker of airway remodeling.

We aimed to examine serum YKL-40 levels and chitotriosidase activity in children with controlled and therapy-resistant asthma, and to investigate potential correlations between YKL-40 levels, bronchial wall thickening as assessed by HRCT, pulmonary function measurements, and other biomarkers of inflammation. Finally, we wanted to examine the effect of genetic variations in the *CHI3L1* and *CHIT1* genes on YKL-40 levels and chitotriosidase activity, respectively. Baseline data on the cohorts of well-characterized asthmatic patients have been published previously.[16]

METHODS

In this cross-sectional, explorative case-control study, children aged 6 to 18 years with problematic severe asthma were compared with age-matched peers with controlled asthma. These 2 conditions were defined in accordance with guidelines established by the Global Initiative for Asthma,[17] which takes into account the severity of the underlying disease, as indicated by the need for treatment and degree of symptom control achieved.[18] Inclusion criteria are presented in Table I. Written informed consent was obtained from all participants and their guardians. The study was approved by the regional board of ethics at Karolinska Institutet (Dnr 2006/1324-31/1 and 2008/378).

Subjects

The children recruited were participating in the Swedish National Study on children with problematic severe asthma. Details of recruitment and inclusion are presented elsewhere.[16] Briefly, allergists throughout Sweden were invited to identify and refer children with problematic severe asthma and age-matched peers (±12 months) with controlled asthma for this investigation. The referred children were invited to participate and following reassessment of their medical histories, medication, and adherence to prescribed treatment, final selection of the participants was performed according to the inclusion criteria (Table I). The children who admitted that they missed more than 3 doses of prescribed medication per week were considered ineligible for inclusion. A mobile team consisting of 2 of the authors (J.K. and B.N.) visited the participating clinics and conducted the interviews and the majority of the procedures on site, using brought equipment, to ensure standardized collection of biological samples and data. As shown in Fig 1, we excluded children who were classified as having asthma that was difficult to treat because of identified aggravating factors (such as known allergen and tobacco exposure, or untreated comorbidities such as rhinitis), described in more detail previously.[16] The reason for removing these children was that they did not necessarily have severe asthma if the aggravating factors were removed/treated.

The remaining 34 children with severe asthma were classified as resistant to therapy on the basis of a detailed clinical characterization.[16] Therefore, the current analysis includes 34 children with therapy-resistant asthma and 39 children with controlled asthma. In addition, we included 27 school-aged healthy controls (average age 10 years) for comparison. These healthy controls had been admitted for elective surgical procedures such as scrotal hernia, and blood samples were collected prior to the procedure.

Procedures

Asthma control was estimated according to the asthma control test.[19] A total of 25 points can be achieved with optimal asthma control, and a score of 19 or less suggests poorly controlled asthma.

Exhaled nitric oxide, pulmonary function, and assessment of bronchial hyperresponsiveness. A NIOX analyzer (Aerocrine AB, Solna, Sweden) was used to measure the fraction of exhaled nitric oxide in exhaled air (FENO), in accordance with international guidelines.[20] Spirometry was performed using a Vitalograph 2120 (Vitalograph, Ennis, Ireland), in accordance with recommendations from the European Respiratory Society using the reference values reported by Polgar and Weng.[21] Bronchial hyperresponsiveness to methacholine was assessed using a Spira nebuliser (Spira Respiratory Care Centre, Hämeenlinna, Finland), and the slope of the dose-response curve was calculated.[22]

CT scanning of lungs. Children suffering from problematic severe asthma underwent HRCT of their lungs at their local hospitals, within 3 months after inclusion into the study. A standardized protocol was used, and all images were evaluated by the same radiologist (M.L.).

HRCT scans were obtained at full inspiration with 1- to 1.5-mm thick sections at 10-mm intervals through the thorax, and images were reconstructed by using a high spatial resolution reconstruction algorithm. The images were viewed at lung window settings optimized for pediatric lungs: window level 500 Hounsfield units and window width 1500 Hounsfield units.

Bronchial wall thickening was assessed using a semiquantitative score first described by Marchac et al[12] and used in previous studies in children with severe asthma.[12,14] The score is based on the assumption that any increase in the thickness of the bronchial wall will be associated with an increase in the number of visible bronchi on HRCT. Consequently, all visible sections of bronchi were counted at 3 predetermined levels in the right lung (1 cm below the carina, 2.5-3 cm below the top of the right pulmonary vein, and above the right side of the diaphragm). All circular (complete circles or at least two thirds of a circle) and longitudinal bronchi except hilar bronchi were included. The number of visible sections of bronchi at these 3 levels were added to yield a score (3-level score) for bronchial wall thickening.[12]

Serologic analysis and number of eosinophils and neutrophils. Following application of local anesthesia (EMLA cream, Astra Zeneca, Sweden), samples of venous blood were collected and an eosinophil and neutrophil count was performed. The serum concentration of IgE (kU/L) was analyzed using ImmunoCAP (Phadia AB, Uppsala, Sweden).

Measurement of chitinase levels. Serum YKL-40 levels were measured by ELISA according to the manufacturer's instructions (Human Chitinase 3-like 1 DuoSet ELISA Development Kit, RnD Systems, Abingdon, United Kingdom). Two different dilutions were made for each sample from which an average was obtained, and all samples were analyzed in duplicate and presented as ng/mL. The detection limit of this assay was 31 pg/mL, interassay variability was 3%, and intraassay variability was 14%. Chitotriosidase activity in the serum was determined by using the fluorogenic substrate 4MU-deoxychitobiose as described and presented as nmol/mL/hour.[23] Further details are provided in the Online Repository at www.jacionline.org.

TABLE I. Criteria for inclusion of children suffering from problematic severe and controlled persistent asthma

Problematic severe asthma	Controlled persistent asthma
Major criteria (all required)	**Major criteria** (all required)
■ A diagnosis of asthma by a pediatric allergist	■ A diagnosis of asthma by a pediatric allergist
■ Daily high-dose administration of ICS (≥800 μg budesonide or ≥400 μg fluticasone/momethasone per day) in combination with LABA and/or LTRA*	■ Daily low- to medium-dose administration of ICS (≥100-≤400 μg budesonide or ≥50-≤200 μg fluticasone per day). Use of either LABA *or* LTRA was acceptable
Minor criteria observed within the preceding 12-mo period (minimum of at least 1 required)	**Minor criteria** observed within the preceding 12-mo period (all required)
■ at least 1 emergency hospitalization	■ no hospitalization
■ at least 2 emergency outpatient visits	■ no emergency outpatient visits
■ at least 1 oral treatment with corticosteroid	■ no oral corticosteroid treatment
■ at least 12 exacerbations of asthmatic symptoms per year or symptoms present continuously for at least 3 mo	■ fewer than 5 exacerbations of symptoms†
■ symptoms that limited daily activities (including sport or leisure activities) more than twice a week for at least 3 consecutive mo	■ occasional symptoms related to strenuous exercise only, otherwise no symptoms
■ nocturnal symptoms more than twice a week for at least 3 consecutive mo	■ no nocturnal symptoms

ICS, Inhaled corticosteroid; *LABA*, long-acting β_2 agonist; *LTRA*, leukotriene receptor antagonist.
*High-dose administration of ICS for at least 6 mo during the preceding year; previous use of LABA or LTRA was considered acceptable only if this treatment was discontinued because of inefficacy or the occurrence of unacceptable side effects.
†An increase in the ICS dosage for a maximum of 2 wk in connection with asthma exacerbations was considered acceptable.

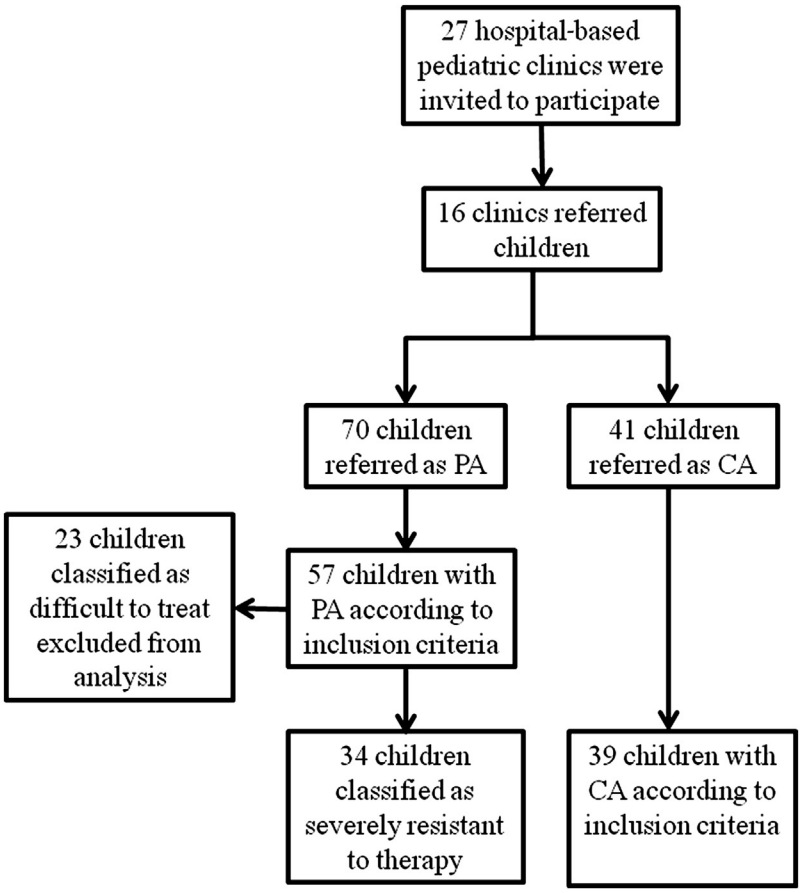


FIG 1. Flow of patients from referral to final inclusion. *CA*, Controlled persistent asthma; *PA*, problematic severe asthma.

Genotyping. DNA was available from all asthmatic children. The -131 C/G polymorphism in the *CHI3L1* gene (rs4950928) was analyzed by using TaqMan allelic discrimination on the ABI Prism 7500 detection system according to manufacturer's protocol (C__27832042_10, Applied Biosystems, Foster City, Calif). The *CHIT1* 24-bp duplication (rs3831317) was genotyped using melting curve analysis to discriminate the different PCR

TABLE II. Comparison of baseline characteristics, asthma control, pulmonary function measurements, and biomarkers of inflammation between children with therapy-resistant asthma and children with controlled asthma

Variable	Therapy-resistant asthma (n = 34)	Controlled asthma (n = 39)	*P* value
Age (y)	13.3 ± 3.1	13.8 ± 2.9	.50
Inhaled corticosteroids (μg budesonide eq), median (IQR)	800 (800-800)	320 (200-400)	NA
Body mass index	20.4 ± 3.5	20.0 ± 3.1	.63
Asthma control test score	17 ± 3.2	23 ± 1.8	<.001
FEV_1 (%)	86 ± 19	90 ± 11	.22
$FEV_1/FVC \times 100$	80 ± 10	85 ± 8	.03
FENO (ppb), median (IQR)	15 (10-38)	17 (9-26)	.93
Dose-response $slope_{methacholine}$, median (IQR)	50 (1.4-92)*	3.4 (0.4-27)	.01
Total IgE (kU/L), median (IQR)	219 (44-885)	280 (66-700)	.92
Blood eosinophils ($10^9 \times L^{-1}$)	0.42 ± 0.4	0.25 ± 0.2	.04
Blood neutrophils ($10^9 \times L^{-1}$)	3.3 ± 1.5	2.7 ± 0.7	.03
YKL-40 (ng/mL), median (IQR)	19.2 (14-23)	16.3 (15-18)	.12
Bronchial wall thickening by HRCT	20.5 ± 8	–†	

Note: All values givens as means ± SD unless otherwise stated.
FVC, Forced vital capacity; *IQR*, interquartile range; *NA*, statistical comparison not relevant because the observed differences reflect the inclusion criteria.
*Dose-response $slope_{methacholine}$: n = 24.
†HRCT not performed on children with controlled asthma.

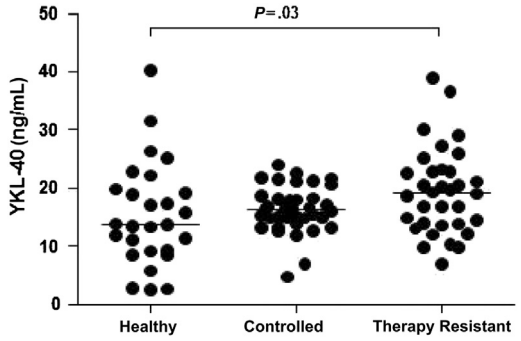


FIG 2. Comparison of serum levels of YKL-40 in children with severe, therapy-resistant asthma, children with controlled persistent asthma, and healthy controls. Results are presented as individual data points with *median bars*.

products based on the presence or absence of the duplication. Details of the primers used are described in the Online Repository at www.jacionline.org. Reruns of 10% of the samples for both genotypes showed 100% concordance.

Statistical analyses

The results presented in this study consisted of data sets with both normal and skewed distributions. Levels of YKL-40 and chitotriosidase activity were presented in figures, and described in the text as medians with interquartile range. The Mann-Whitney test was used to analyze between-group differences in chitinases, IgE, dose-response slope for methacholine, and FENO, whereas Student *t* test was used to analyze differences in asthma control test scores, body mass index, quantification of bronchial wall thickening, pulmonary function measurements, and white blood cells count. Correlations were assessed using the Pearson test or the Spearman test depending on the distribution of the 2 data sets being investigated, and a χ^2 test was used to test associations between categorical variables. All values were analyzed with the IBM SPSS Statistics (Chicago, Ill), Version 20.0.

The high number of hypothesis tests performed in this study is by definition associated with the risk of a high number of false positives ($80 \times 0.05 = 4$ false positives on average). Correction for multiple testing was therefore performed according to the method of Benjamini and Hochberg.[24] Applying a false-discovery rate of 5% (.05) corresponds to a raw *P* value of .011. However, using false-discovery rate *P*-value correction to reduce the number of false positives comes with the penalty of a drastic decrease in statistical power. As such, both *P* values and the corresponding false-discovery rate values are reported for all presented results (see Table E1 in this article's Online Repository at www.jacionline.org).

RESULTS

Clinical characteristics of the included patients are presented in Table II. Children with therapy-resistant asthma (n = 34) received higher doses of inhaled corticosteroids, had inferior asthma control ($P < .001$), more airflow obstruction ($P = .03$), increased bronchial hyperresponsiveness ($P = .01$), and increased numbers of blood eosinophils ($P = .04$) and neutrophils ($P = .03$) compared to children with controlled asthma (n = 39). There were no differences between these 2 groups of children with respect to age at inclusion, body mass index, FENO, and total IgE in blood.

Serum levels of YKL-40 in children with severe, therapy-resistant asthma, children with controlled asthma, and healthy controls

YKL-40 levels (ng/mL) were measured in serum from children with severe, therapy-resistant asthma (n = 34), children with controlled asthma (n = 39), and healthy controls (n = 27) (Table II and Fig 2). Significantly higher levels of YKL-40 were found in serum from children with severe, therapy-resistant asthma compared with healthy children (19.2 [14-23] vs 13.8 [9-20], $P = .03$). The differences between children with therapy-resistant and children with controlled asthma (19.2 [14-23] vs 16.3 [15-18], $P = .12$) and between children with controlled asthma and healthy controls ($P = .26$) were not statistically significant.

Associations between YKL-40 levels, asthma control, bronchial wall thickening, and biomarkers of inflammation

Among all children with asthma, there were correlations between YKL-40 levels and asthma control ($r = -0.38$, $P = .001$) and blood neutrophils ($r = 0.51$, $P < .001$). These correlations were also evident in children with severe, therapy-resistant asthma (n = 34): asthma control ($r = -0.38$, $P = .03$) and blood

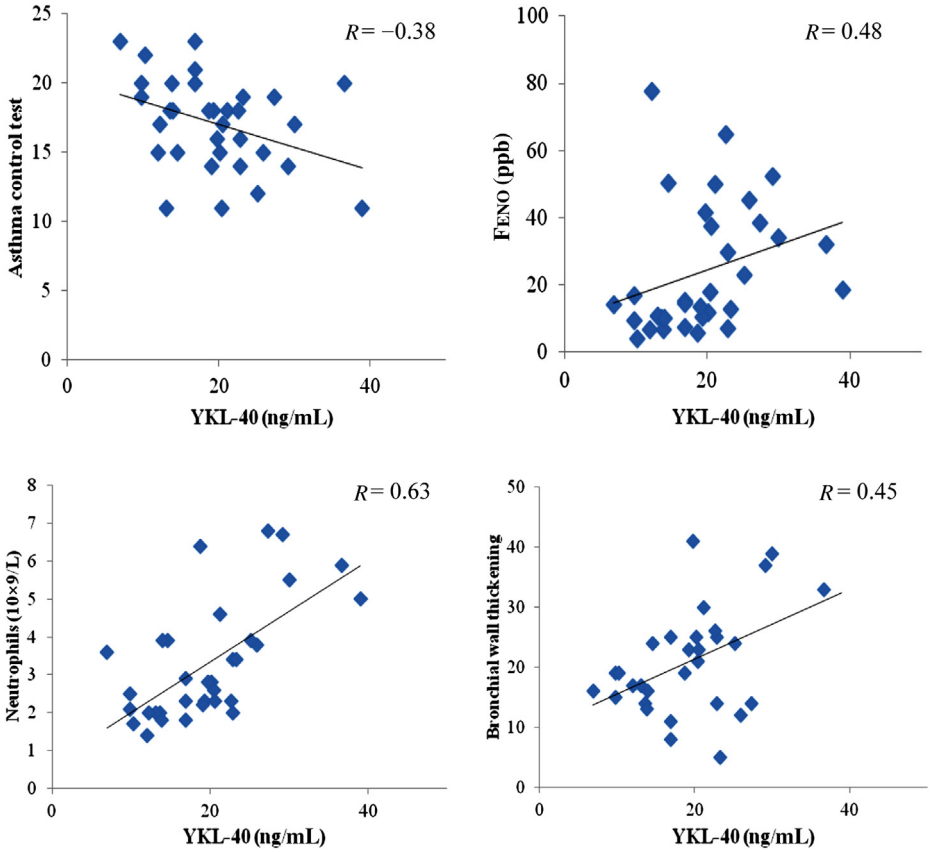


FIG 3. Correlations between YKL-40 levels, asthma control, FENO, neutrophils, and bronchial wall thickening among children with severe, therapy-resistant asthma.

TABLE III. Comparison of asthmatic children with YKL-40 levels above the median (16.9 ng/mL) to children with YKL-40 levels below the median

Variable	YKL-40 levels above the median (n = 36)	YKL-40 levels below the median (n = 36)	*P* value
Age (y)	14.3 ± 2.7	12.8 ± 3.6	.04
Inhaled corticosteroids (μg budesonide eq), median (IQR)	800 (320-800)	400 (200-800)	.12
Asthma control test	18.8 ± 3.9	21.6 ± 3.4	.002
FEV_1 (%)	91 ± 15	85 ± 15	.13
FENO (ppb), median (IQR)	18.9 (12-38)	14 (9-20)	.045
Dose-response $slope_{methacholine}$, median (IQR)*	4.2 (0.5-45)	11.3 (0.6-52)	.63
Blood eosinophils ($10^9 \times L^{-1}$)	0.31 ± 0.2	0.35 ± 0.4	.60
Blood neutrophils ($10^9 \times L^{-1}$)	3.3 ± 1.4	2.6 ± 0.8	.01
Bronchial wall thickening on HRCT†	23.2 ± 8.6	16.9 ± 5.8	.03

Note: All values givens as means ± SD unless otherwise stated.
IQR, Interquartile range.
*Dose-response $slope_{methacholine}$: n = 33 below median and n = 28 above median.
†Bronchial wall thickening: n = 18 above median and n = 13 below median.

neutrophils ($r = 0.63$, $P < .001$) (Fig 3). Furthermore, among children with therapy-resistant asthma, there were significant correlations between YKL-40 levels and FENO ($r = 0.48$, $P = .004$), as well as with airway remodeling assessed by bronchial wall thickening on HRCT ($r = 0.45$, $P = .01$; Fig 3). There were no significant correlations between YKL-40 levels with measurements of pulmonary function or bronchial hyperresponsiveness (data not shown). When investigating all children with asthma (n = 73), those with YKL-40 levels above the median (16.9 ng/mL) were older ($P = .04$) and had reduced asthma control ($P = .002$), increased bronchial wall thickening on HRCT ($P = .03$), more blood neutrophils ($P = .01$), and higher FENO ($P = .045$) compared to those with YKL-40 levels below the median (Table III).

Associations between the -131 C/G polymorphism (rs4950928) in *CHI3L1*, YKL-40 levels, and asthma severity

Children homozygous for the C allele (n = 46) had higher circulating levels of YKL-40 (18.3 [16-23]), compared to heterozygous (CG) carriers (n = 24, 13.7 [12-16]; $P < .001$, Fig 4). Fifty-five percent of the children with therapy-resistant asthma

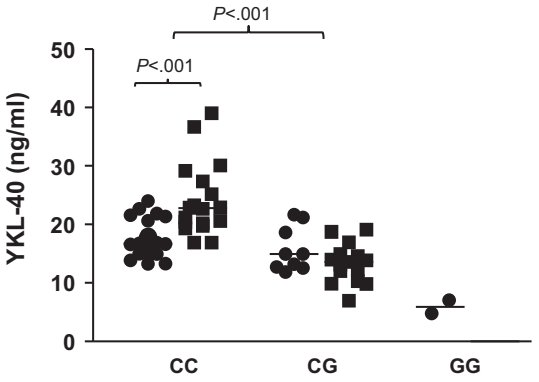


FIG 4. The effect of genetic variability on chitinase levels in all asthmatic subjects. Serum YKL-40 levels are categorized according to *CHI3L1* genotype (rs4950928) and asthma severity. Results from children with controlled asthma (●) and children with severe, therapy-resistant asthma (■) are displayed as individual data points with *median bars*.

were homozygous for the C allele (n = 18) compared with 72% of the children with controlled asthma ($P = .07$). Among the CC carriers, children with severe, therapy-resistant asthma had higher levels of YKL-40 compared to those with controlled asthma (22.8 [20-28] vs 16.7 [15 -18]; $P < .001$, Fig 4). Among the CG carriers, there was no significant difference in YKL-40 levels between children with therapy-resistant asthma and children with controlled asthma. The number of subjects homozygous for the G allele is too small for between-group comparisons.

Associations between the CHI3L1-131 C/G polymorphism (rs4950928) and clinical characteristics

There was no difference between CC carriers and CG carriers with respect to, age, body mass index, asthma control, bronchial wall thickening, or pulmonary function measurements (data not shown). However, CC carriers had higher levels of blood neutrophils (3.2 vs 2.5, $P = .04$). In addition, CC carriers with therapy-resistant asthma had higher levels of FENO compared to CG carriers with therapy-resistant asthma (26 [14-39] vs 10 [7-14], $P = .003$). Among all CC carriers, there were correlations between YKL-40 levels and asthma control ($r = -0.59$, $P < .001$), FEV_1 ($r = 0.34$, $P = .02$), neutrophils ($r = 0.6$, $P < .001$), FENO ($r = 0.48$, $P = .001$), and bronchial wall thickening (r = 0.58, $P = .02$). Among CG carriers, none of these correlations were found (results not shown).

Serum levels of chitotriosidase activity in children with severe, therapy-resistant asthma, children with controlled asthma, and healthy controls and associations with the *CHIT1* 24-bp duplication (rs3831317)

Chitotriosidase activity (nmol/mL/h) was not significantly higher in serum from children with severe, therapy-resistant asthma (57.0 [36-83]) compared to those with controlled asthma (52.0 [36-60], $P = .22$), or compared with healthy controls (59.0 [41-68], $P = .26$; see Fig E1 in this article's Online Repository at www.jacionline.org). Children without the 24-bp duplication in exon 10 (75/75 bp homozygous) had significantly higher chitotriosidase activity compared with those heterozygous for the duplication (75/99 heterozygous) (63.5 [52-82] vs 36.5 [29-43], $P < .001$; see Fig E2 in this article's Online Repository at www.jacionline.org). Among the 75/75 homozygous children, those with severe, therapy-resistant asthma had higher levels of chitotriosidase activity compared to those with controlled asthma (75.5 [54-102] vs 58.0 [52-73], $P = .03$).

DISCUSSION

In this cohort of well-characterized school-aged children with asthma, we demonstrate associations between YKL-40 levels, clinical characteristics, and disease severity. We show for the first time that serum YKL-40 level is increased in children with severe, therapy-resistant asthma compared with healthy children. Furthermore, serum levels of YKL-40 significantly correlate with asthma control, airway remodeling measured as bronchial wall thickening by HRCT, and blood neutrophils in children with severe resistance to therapy. Moreover, 2 entirely new insights were made by exploring the association between YKL-40 levels and asthma severity in relation to the natural variability in chitinase levels caused by a common single nucleotide polymorphism (rs4950928) in *CHI3L1*. First, among children without this specific mutation in CHI3L1 (CC carriers), those with therapy-resistant asthma had higher levels of YKL-40 compared with those with controlled asthma. Second, among heterozygous children (CG carriers), no difference in YKL-40 levels was found between those with therapy-resistant asthma and those with controlled asthma. A particular strength of the present study is that we have investigated only children with severe, therapy-resistant asthma and have excluded children classified as difficult to treat, that is, children whose lack of asthma control could be explained by complex problems such as poor adherence, comorbidity, environmental exposures (allergens, tobacco), or psychological issues. Severe therapy resistance was diagnosed when a detailed and standardized workup failed to identify any underlying problem, but the child still suffered from chronic symptoms and/or recurrent exacerbations, as described previously in more detail.[16] Accordingly, children with therapy-resistant asthma have truly severe asthma, and therefore it is more relevant to study biomarkers related to asthma severity in this group.

Several members of the glycosyl hydrolase family 18 of chitinases are expressed in humans, of which YKL-40, chitotriosidase, and acidic mammalian chitinase have been linked to airway inflammation.[11] In the current study, we focused on YKL-40 as this protein has been suggested to be a biomarker of severe asthma in adults.[8] We found that children with severe therapy resistance had significantly higher levels of YKL-40 compared with healthy controls, whereas no difference was found between children with controlled asthma and healthy controls. Children with controlled asthma displayed intermediate YKL-40 levels between those of the healthy controls and those of therapy-resistant asthmatic children. These results, together with the inverse correlation between YKL-40 levels and asthma control, strengthen the view that increased levels of YKL-40 in children are associated with severe asthma, and extend previous observations made in Chinese, French, and North American cohorts of adult patients with asthma.[8,25] The nonsignificant difference in YKL-40 levels between children with therapy-resistant asthma and children with controlled asthma in the initial group comparison could be caused by insufficient statistical power, which is one of the limitations of this study.

In our analysis of chitotriosidase activity in children with controlled asthma and children with therapy-resistant asthma and

healthy volunteers, we found no between-group differences. Previous reports regarding chitotriosidase activity in asthma are few and conflicting, and none have been performed in children. Chitotriosidase activity has been reported to be reduced in asthma,[26] shown to be no different compared with that of healthy controls,[27] as well as increased in asthmatic subjects,[7] and further studies are required to clarify a possible role.

One approach taken to examine which clinical characteristics are associated with high serum YKL-40 levels was to compare asthmatic children with YKL-40 levels above and below the median of the whole group. These results showed that children with high levels of YKL-40 were older and had reduced asthma control, increased bronchial wall thickening on HRCT, more blood neutrophils in blood, and higher FENO compared with those with YKL-40 levels below the median, thus revealing a cluster of clinical and pathophysiologic variables associated with high levels of YKL-40.

The finding of a correlation between serum YKL-40 levels and bronchial wall thickening in children with therapy-resistant asthma is of particular interest. The image of thickened bronchial walls represents not only an increased thickness of the reticular membrane but also hypertrophy and hyperplasia of the bronchial smooth muscle, myofibroblastic proliferation, increased numbers and size of the mucus glands, and angiogenesis.[28] Our findings strengthen the view that YKL-40 is a marker of or directly involved in remodeling of the airways. Chupp et al[8] found a correlation between serum and bronchoalveolar lavage levels of YKL-40 and subepithelial basal membrane thickness. Gavala et al[29] performed an allergen challenge in patients with mild asthma and report increased YKL-40 levels in bronchoalveolar lavage 48 hours postchallenge. They also found correlations between YKL-40 levels and profibrotic factors, which also support the involvement of chitinases in airway remodeling. YKL-40 binds to collagen I and can regulate the formation of collagen fibrils[30] and has recently been shown to increase the proliferation and migration of human bronchial smooth muscle.[31] In relation to other diseases also characterized by chronic inflammation and tissue remodeling, YKL-40 has been shown to play a role in fibrosis and angiogenesis by regulating the survival, adhesion, migration, and proliferation of various structural cell types.[11]

Another noteworthy finding is the close correlation between YKL-40 levels and blood neutrophils. Previous studies have indicated that alveolar macrophages are a major source of YKL-40 in the airways,[32] although it is known that YKL-40 can be stored in the specific granules of neutrophils and released upon activation of these cells.[33] A recent study of sputum YKL-40 levels in adults with asthma also demonstrated a strong correlation with sputum neutrophil count[34] in agreement with the findings of the current study.

Variation in the *CHI3L1* gene was strongly associated with serum YKL-40 levels as children homozygous for the C allele had higher levels of YKL-40 than did CG carriers. The investigated single nucleotide polymorphism (rs4950928) is in the promoter for *CHI3L1*. The G allele disrupts the binding of transcription factors and is associated with lower levels of messenger RNA, lower YKL-40 levels,[35] improved pulmonary function, lower prevalence of asthma,[9] and a reduced number of asthma-related hospital admission.[36] A novel finding in the current study is that the G allele is associated with lower numbers of neutrophils and lower FENO.

Among CC carriers, children with severe, therapy-resistant asthma had significantly higher levels of YKL-40 compared to those with controlled asthma. This finding was supported by stronger correlations between YKL-40 levels and asthma control, pulmonary function measurement, airway remodeling, and inflammatory biomarkers among CC carriers. Among the CG carriers, we found no difference in YKL-40 levels between those with therapy-resistant asthma and those with controlled asthma and none of the above-mentioned correlations were found. Accordingly, the association between YKL-40 levels and asthma severity is most evident among CC carriers who have the capacity to produce higher levels of YKL-40, an association that may be less obvious when analyses include CG carriers or GG carriers who produce lower levels of YKL-40. These findings demonstrate that the natural variability in YKL-40 levels caused by common mutations in *CHI3L1* may need to be considered if YKL-40 is to be used as a marker of asthma severity. YKL-40 in the airways is a marker of and/or is involved in airway remodeling, and the increased levels of YKL-40 observed in those with severe asthma may be due to the greater degree of airway remodeling in these patients. Little is currently known about the genetic causes of severe, therapy-resistant asthma in children,[37] and our data support a role of genes regulating YKL-40 levels in disease development.

In conclusion, children with severe, therapy-resistant asthma have higher levels of YKL-40 than do healthy controls, and adjustment for genotype also revealed higher levels in those with therapy-resistant asthma compared with those with controlled asthma. Our results demonstrate that high levels of YKL-40 in asthmatic children are associated with poor asthma control, increased markers of inflammation, and remodeling as well as variations in *CHI3L1*. Taken together, these findings indicate that YKL-40 may be an easily attainable biomarker of asthma severity and airway remodeling in children.

We acknowledge the contribution from members of The Swedish Network of Pediatric Allergists working with severe asthma, who helped in identifying patients for this study. The network consists of the following individuals: Helen Axfors Olsson, Växjö General Hospital; Magnus Aurivillius, Malmö General Hospital; Carl Axel Hederos and Lars Häggblom, Karlstad General Hospital; Anna Hedlund and Stig Örjestad, Skellefteå General Hospital; Anders Lindfors, Maria Ingemansson, Louise Lindgren, Sten-Erik Bergström, Anne Kihlström, Päivi Söderman, Awder Mustafa, and Mia Stalberg, Karolinska University Hospital; Eva Landgren, Sundsvall General Hospital; Lennart Nordvall, Pia Kalm-Stephens, and Fredrik Cederblad, Uppsala University Hospital; Jan Svedmyr, Falun General Hospital; Per Thunqvist, Sachsska Children's Hospital; Göran Wennergren, Bill Hesselmar, and Nils Åberg, Sahlgrenska University Hospital; Anna Winberg, Norrland University Hospital; Kajsa Öberg and Olaf Küssner, Norra Älvsborg General Hospital; Nils-Olof Jonsson and Göran Oldeus Ryhov, County Hospital of Jönköping; and Lennart Nilsson, Regional Hospital of Linköping.

Clinical implications: Serum YKL-40 level may be a new biomarker of airway inflammation and remodeling in children with severe asthma.

REFERENCES

1. Bousquet J, Mantzouranis E, Cruz AA, Ait-Khaled N, Baena-Cagnani CE, Bleecker ER, et al. Uniform definition of asthma severity, control, and exacerbations: document presented for the World Health Organization Consultation on Severe Asthma. J Allergy Clin Immunol 2010;126:926-38.
2. Fitzpatrick AM, Gaston BM, Erzurum SC, Teague WG. Features of severe asthma in school-age children: atopy and increased exhaled nitric oxide. J Allergy Clin Immunol 2006;118:1218-25.
3. Bush A, Hedlin G, Carlsen KH, de Benedictis F, Lodrup-Carlsen K, Wilson N. Severe childhood asthma: a common international approach? Lancet 2008;372:1019-21.

4. Fitzpatrick AM, Teague WG. Severe asthma in children: insights from the National Heart, Lung, and Blood Institute's Severe Asthma Research Program. Pediatr Allergy Immunol Pulmonol 2010;23:131-8.
5. Lodrup Carlsen KC, Hedlin G, Bush A, Wennergren G, de Benedictis FM, De Jongste JC, et al. Assessment of problematic severe asthma in children. Eur Respir J 2011;37:432-40.
6. Bussink AP, Speijer D, Aerts JM, Boot RG. Evolution of mammalian chitinase (like) members of family 18 glycosyl hydrolases. Genetics 2007;177:959-70.
7. Bargagli E, Olivieri C, Margollicci M, Bennett D, Luddi A, Perrone M, et al. Serum chitotriosidase levels in patients with allergic and non-allergic asthma. Respiration 2010;79:437-8.
8. Chupp GL, Lee CG, Jarjour N, Shim YM, Holm CT, He S, et al. A chitinase-like protein in the lung and circulation of patients with severe asthma. N Engl J Med 2007;357:2016-27.
9. Ober C, Tan Z, Sun Y, Possick JD, Pan L, Nicolae R, et al. Effect of variation in CHI3L1 on serum YKL-40 level, risk of asthma, and lung function. N Engl J Med 2008;358:1682-91.
10. Boot RG, Renkema GH, Verhoek M, Strijland A, Bliek J, de Meulemeester TM, et al. The human chitotriosidase gene: nature of inherited enzyme deficiency. J Biol Chem 1998;273:25680-5.
11. Lee CG, Da Silva CA, Dela Cruz CS, Ahangari F, Ma B, Kang MJ, et al. Role of chitin and chitinase/chitinase-like proteins in inflammation, tissue remodeling, and injury. Annu Rev Physiol 2011;73:479-501.
12. Marchac V, Emond S, Mamou-Mani T, Le Bihan-Benjamin C, Le Bourgeois M, De Blic J, et al. Thoracic CT in pediatric patients with difficult-to-treat asthma. AJR Am J Roentgenol 2002;179:1245-52.
13. Kasahara K, Shiba K, Ozawa T, Okuda K, Adachi M. Correlation between the bronchial subepithelial layer and whole airway wall thickness in patients with asthma. Thorax 2002;57:242-6.
14. de Blic J, Tillie-Leblond I, Emond S, Mahut B, Dang Duy TL, Scheinmann P. High-resolution computed tomography scan and airway remodeling in children with severe asthma. J Allergy Clin Immunol 2005;116:750-4.
15. Aysola RS, Hoffman EA, Gierada D, Wenzel S, Cook-Granroth J, Tarsi J, et al. Airway remodeling measured by multidetector CT is increased in severe asthma and correlates with pathology. Chest 2008;134:1183-91.
16. Konradsen JR, Nordlund B, Lidegran M, Pedroletti C, Gronlund H, van Hage M, et al. Problematic severe asthma: a proposed approach to identifying children who are severely resistant to therapy. Pediatr Allergy Immunol 2011;22:9-18.
17. Global Initiative for Asthma (GINA). From the Global Strategy for Asthma Management and Prevention. 2012. Available from: www.ginasthma.org. Accessed January 5, 2013.
18. Taylor DR, Bateman ED, Boulet LP, Boushey HA, Busse WW, Casale TB, et al. A new perspective on concepts of asthma severity and control. Eur Respir J 2008;32:545-54.
19. Nathan RA, Sorkness CA, Kosinski M, Schatz M, Li JT, Marcus P, et al. Development of the asthma control test: a survey for assessing asthma control. J Allergy Clin Immunol 2004;113:59-65.
20. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med 2005;171:912-30.
21. Polgar G, Weng TR. The functional development of the respiratory system from the period of gestation to adulthood. Am Rev Respir Dis 1979;120:625-95.
22. O'Connor G, Sparrow D, Taylor D, Segal M, Weiss S. Analysis of dose-response curves to methacholine: an approach suitable for population studies. Am Rev Respir Dis 1987;136:1412-7.
23. Schoonhoven A, Rudensky B, Elstein D, Zimran A, Hollak CE, Groener JE, et al. Monitoring of Gaucher patients with a novel chitotriosidase assay. Clin Chim Acta 2007;381:136-9.
24. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B 1995;57:289-300.
25. Tang H, Fang Z, Sun Y, Li B, Shi Z, Chen J, et al. YKL-40 in asthmatic patients, and its correlations with exacerbation, eosinophils and immunoglobulin E. Eur Respir J 2010;35:757-60.
26. Seibold MA, Donnelly S, Solon M, Innes A, Woodruff PG, Boot RG, et al. Chitotriosidase is the primary active chitinase in the human lung and is modulated by genotype and smoking habit. J Allergy Clin Immunol 2008;122:944-50.e3.
27. Tercelj M, Salobir B, Simcic S, Wraber B, Zupancic M, Rylander R. Chitotriosidase activity in sarcoidosis and some other pulmonary diseases. Scand J Clin Lab Invest 2009;69:575-8.
28. de Blic J, Scheinmann P. The use of imaging techniques for assessing severe childhood asthma. J Allergy Clin Immunol 2007;119:808-10.
29. Gavala ML, Kelly EAB, Esnault S, Kukreja S, Evans MD, Bertics PJ, et al. Segmental allergen challenge enhances chitinase activity and levels of CCL18 in mild atopic asthma (pages 187-197). Clin Exp Allergy 2013;43:187-97.
30. Bigg HF, Wait R, Rowan AD, Cawston TE. The mammalian chitinase-like lectin, YKL-40, binds specifically to type I collagen and modulates the rate of type I collagen fibril formation. J Biol Chem 2006;281:21082-95.
31. Bara I, Ozier A, Girodet PO, Carvalho G, Cattiaux J, Begueret H, et al. Role of YKL-40 in bronchial smooth muscle remodeling in asthma. Am J Respir Crit Care Med 2012;185:715-22.
32. Letuve S, Kozhich A, Arouche N, Grandsaigne M, Reed J, Dombret MC, et al. YKL-40 is elevated in patients with chronic obstructive pulmonary disease and activates alveolar macrophages. J Immunol 2008;181:5167-73.
33. Volck B, Price PA, Johansen JS, Sorensen O, Benfield TL, Nielsen HJ, et al. YKL-40, a mammalian member of the chitinase family, is a matrix protein of specific granules in human neutrophils. Proc Assoc Am Phys 1998;110: 351-60.
34. Otsuka K, Matsumoto H, Niimi A, Muro S, Ito I, Takeda T, et al. Sputum YKL-40 levels and pathophysiology of asthma and chronic obstructive pulmonary disease. Respiration 2012;83:507-19.
35. Zhao X, Tang R, Gao B, Shi Y, Zhou J, Guo S, et al. Functional variants in the promoter region of Chitinase 3-like 1 (CHI3L1) and susceptibility to schizophrenia. Am J Hum Genet 2007;80:12-8.
36. Cunningham J, Basu K, Tavendale R, Palmer CN, Smith H, Mukhopadhyay S. The CHI3L1 rs4950928 polymorphism is associated with asthma-related hospital admissions in children and young adults. Ann Allergy Asthma Immunol 2011; 106:381-6.
37. Melen E, Pershagen G. Pathophysiology of asthma: lessons from genetic research with particular focus on severe asthma. J Intern Med 2012;272:108-20.

METHODS

Measurement of chitotriosidase activity and analysis of CHIT1 rs3831317 genotype

Chitotriosidase activity in the serum was determined by using the fluorogenic substrate 4MU-deoxychitobiose as described and presented as nmol/mL/h. Briefly: 25 μL of serum, diluted with BSA/PBS (1 mg/mL) and 100 μL of substrate mixture, 0.111 mmol/L 4MU-deoxychitobiose, and 1 mg/mL of BSA in McIlvain buffer (100 mmol/L citric acid, 200 mmol/L sodium phosphate) (pH 5.2) were incubated for 20 minutes at 37°C. Reactions were stopped with 2.0 mL of 0.3 mol/L glycine NaOH buffer (pH 10.6), and the formed 4MU was detected fluorometrically (excitation at 445 nm; emission at 366 nm). To discriminate between chitotriosidase activity and that of acidic mammalian chitinase in serum samples, a neutralizing antibody against acidic mammalian chitinase was used.

The *CHIT1* 24-bp duplication (rs3831317) was genotyped by using melting curve analysis to discriminate the different PCR products on the basis of the presence or absence of the duplication. The following forward (5′-GGAGAAGCCGGCAAAGTC-3′) and reverse primers (5′-AGCTATCTGAAGCAGAAG-3′) were used. PCR reactions were performed with 10 μmol/L of each primer, in a final volume of 10 μL, using SYBR Green PCR Master Mix (Applied Biosystems) following the manufacturer's recommended protocol. Melting curve analysis was performed as a post-PCR procedure with the SDS v1.4 software using the ABI Prism 7500 detection system (Applied Biosystems).

Correction for multiple statistical analyses

Correction for multiple testing was performed according to the method of Benjamini and Hochberg.[24] *P* values and corresponding false-discovery rate values are reported for all presented results in Table E1.

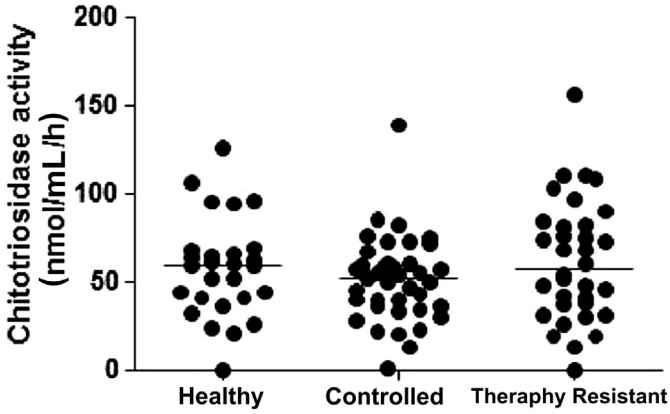


FIG E1. Comparison of serum levels of chitotriosidase activity in children with severe, therapy-resistant asthma, children with controlled persistent asthma, and healthy controls. Results are presented as individual data points with *median bars*.

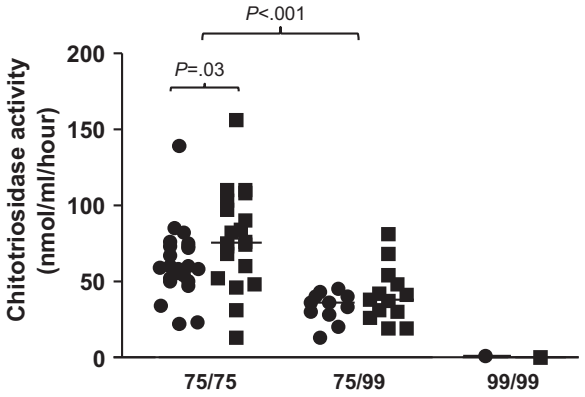


FIG E2. The effect of CHIT1 rs 3831317 genotype on serum chitotriosidase activity in all asthmatic subjects. The *CHIT1* 24-bp duplication (rs3831317) results in a nonfunctional protein lacking enzymatic activity. Children were grouped into those lacking the 24-bp duplication in exon 10 (75/75 bp homozygous, wild-type), heterozygote carriers (75/99 bp), and those homozygous for the duplication (99/99 bp). Results from children with controlled asthma (●) and children with severe, therapy-resistant asthma (■) are displayed as individual data points with *median bars*.

TABLE E1. Benjamini and Hochberg[24] correction for multiple testing

	Variable	Patient group 1/variable	Patient group 2	Test	*P* value	FDR
1	Asthma control test	Therapy resistant	Controlled asthma	*t* Test	.001	.008
2	YKL-40	CC	CG	Mann-Whitney	.001	.008
3	YKL-40	CC: Therapy resistant	CC: controlled asthma	Mann-Whitney	.001	.008
4	Chitotriosidase	75/75	75/99	Mann-Whitney	.001	.008
5	YKL-40	Asthma control	All children with asthma	Pearson/Spearman	.001	.008
6	YKL-40	Blood neutrophils	All children with asthma	Pearson/Spearman	.001	.008
7	YKL-40	Blood neutrophils	Therapy resistant	Pearson/Spearman	.001	.008
8	YKL-40	Asthma control	CC	Pearson/Spearman	.001	.008
9	YKL-40	Neutrophils	CC	Pearson/Spearman	.001	.008
10	YKL-40	FENO	CC	Pearson/Spearman	.001	.008
11	Asthma control test	YKL-40 > median	YKL-40 < median	*t* Test	.002	.01
12	FENO	CC Therapy resistant	CG therapy resistant	Mann-Whitney	.003	.02
13	YKL-40	FENO	Therapy resistant	Pearson/Spearman	.004	.02
14	Chitotriosidase	Blood neutrophils	All children with asthma	Pearson/Spearman	.007	.04
15	Methacholine	Therapy resistant	Controlled asthma	Mann-Whitney	.01	.04
16	Blood neutrophils	YKL-40 > median	YKL-40 < median	*t* Test	.01	.04
17	YKL-40	HRCT	All children with asthma	Pearson/Spearman	.01	.04
18	YKL-40	HRCT	Therapy resistant	Pearson/Spearman	.01	.04
19	YKL-40	FEV_1	CC	Pearson/Spearman	.02	.08
20	YKL-40	Bronchial wall thickening	CC	Pearson/Spearman	.02	.08
21	$FEV_1/FVC \times 100$	Therapy resistant	Controlled asthma	*t* Test	.03	.09
22	Blood neutrophils	Therapy resistant	Controlled asthma	*t* Test	.03	.09
23	Bronchial wall thickening	YKL-40 > median	YKL-40 < median	*t* Test	.03	.09
24	YKL-40	Therapy resistant	Healthy controls	Mann-Whitney	.03	.09
25	Chitotriosidase	75/75 Therapy resistant	75/75 Controlled asthma	Mann-Whitney	.03	.09
26	YKL-40	Asthma control	Therapy resistant	Pearson/Spearman	.03	.09
27	Chitotriosidase	Blood neutrophils	Therapy resistant	Pearson/Spearman	.03	.09
28	Blood eosinophils	Therapy resistant	Controlled asthma	*t* Test	.04	.10
29	Age	YKL-40 > median	YKL-40 < median	*t* Test	.04	.10
30	Blood neutrophils	CC	CG	*t* Test	.04	.10
31	Chitotriosidase	Asthma control	All children with asthma	Pearson/Spearman	.04	.10
32	FENO	YKL-40 > median	YKL-40 < median	Mann-Whitney	.045	.11
33	YKL-40	FENO	All children with asthma	Pearson/Spearman	.07	.16
34	Therapy resistant with CC compared with controlled asthma with CC			χ^2	.07	.16
35	Chitotriosidase	Asthma control	Therapy resistant	Pearson/Spearman	.11	.25
36	YKL-40	Therapy resistant	Controlled asthma	Mann-Whitney	.12	.26
37	Inhaled corticosteroids	YKL-40 > median	YKL-40 < median	Mann-Whitney	.12	.26
38	FEV_1	YKL-40 > median	YKL-40 < median	*t* Test	.13	.27
39	Chitotriosidase	FENO	All children with asthma	Pearson/Spearman	.14	.29
40	YKL-40	Bronchial wall thickening	CG	Pearson/Spearman	.18	.36
41	YKL-40	CG: therapy resistant	CG: controlled asthma	Mann Whitney	.19	.37
42	FEV_1	Therapy resistant	Controlled asthma	*t* Test	.22	.41
43	Chitotriosidase	Therapy resistant	Controlled asthma	Mann-Whitney	.22	.41
44	Bronchial wall thickening	CC	CG	*t* Test	.24	.44
45	Chitotriosidase	Therapy resistant	Healthy controls	Mann-Whitney	.26	.45
46	YKL-40	Controlled asthma	Healthy controls	Mann-Whitney	.26	.45
47	YKL-40	Methacholine	Therapy resistant	Pearson/Spearman	.27	.46
48	Chitotriosidase	Blood eosinophls	All children with asthma	Pearson/Spearman	.29	.46
49	YKL-40	FEV_1	Therapy resistant	Pearson/Spearman	.29	.46
50	Chitotriosidase	FEV_1	Therapy resistant	Pearson/Spearman	.29	.46
51	Chitotriosidase	FEV_1	All children with asthma	Pearson/Spearman	.3	.47
52	Asthma control test	CC	CG	*t* Test	.307	.47
53	Chitotriosidase	HRCT	All children with asthma	Pearson/Spearman	.34	.60
54	Chitotriosidase	HRCT	Therapy resistant	Pearson/Spearman	.34	.50
55	YKL-40	Methacholine	All children with asthma	Pearson/Spearman	.38	.55
56	YKL-40	Blood eosinophls	All children with asthma	Pearson/Spearman	.39	.55
57	YKL-40	FEV_1	CG	Pearson/Spearman	.39	.55
58	YKL-40	Blood eosinophls	Therapy resistant	Pearson/Spearman	.42	.58
59	Blood eosinophils	CC	CG	*t* Test	.47	.64
60	YKL-40	Neutrophils	CG	Pearson/Spearman	.49	.65
61	Age	Therapy resistant	Controlled asthma	*t* Test	.5	.65
62	YKL-40	FEV_1	All children with asthma	Pearson/Spearman	.5	.65

(Continued)

TABLE E1. *(Continued)*

	Variable	Patient group 1/variable	Patient group 2	Test	*P* value	FDR
63	Blood eosinophils	YKL-40 > median	YKL-40 < median	*t* Test	.6	.75
64	Chitotriosidase	Controlled asthma	Healthy controls	Mann-Whitney	.6	.75
65	Body mass index	Therapy resistant	Controlled asthma	*t* Test	.63	.75
66	Methacholine	YKL-40 > median	YKL-40 < median	Mann-Whitney	.63	.75
67	Chitotriosidase	Methacholine	Therapy resistant	Pearson/Spearman	.63	.75
68	$FEV_1/FVC \times 100$	YKL-40 > median	YKL-40 < median	*t* Test	.66	.78
69	75/75: Therapy resistant vs controlled			χ^2	.69	.8
70	Chitotriosidase	Blood eosinophils	Therapy resistant	Pearson/Spearman	.74	.83
71	Chitotriosidase	FENO	Therapy resistant	Pearson/Spearman	.74	.83
72	YKL-40	Asthma control	CG	Pearson/Spearman	.77	.86
73	FEV_1	CC	CG	*t* Test	.83	.91
74	BMI	CC	CG	*t* Test	.87	.93
75	Age	CC	CG	*t* Test	.88	.93
76	Chitotriosidase	Methacholine	All children with asthma	Pearson/Spearman	.88	.93
77	YKL-40	FENO	CG	Pearson/Spearman	.9	.94
78	Total IgE	Therapy resistant	Controlled asthma	Mann-Whitney	.92	.94
79	FENO	Therapy resistant	Controlled asthma	Mann-Whitney	.93	.94
80	Chitotriosidase	75/99 Therapy resistant	75/99 Controlled asthma	Mann-Whitney	.96	.96

Note: *P* value/FDR conversion (HT = 80): *P*, .05 (FDR = .13); *P*, .011 (FDR = .05).
BMI, Body mass index; *FDR*, false-discovery rate; *FVC*, forced vital capacity; *HT*, hypothesis tests.