

Novel severe wheezy young children phenotypes: Boys atopic multiple-trigger and girls nonatopic uncontrolled wheeze

Jocelyne Just, PhD, MD,^a Rahele Gouvis-Echraghi, MD,^a Remy Couderc, PhD, PharmD,^b
Nathalie Guillemot-Lambert, MD,^a and Philippe Saint-Pierre, PhD^c Paris, France

Background: Recurrent wheezing during infancy is a heterogeneous disorder that has been associated with early-onset asthma.

Objective: To identify phenotypes of severe recurrent wheezing and therapeutic approaches.

Methods: We performed cluster analysis with 20 variables of 551 children with active asthma, younger than 36 months old, and enrolled in the Trousseau Asthma Program.

Results: We identified 3 independent clusters of children with wheezing. Cluster 1, mild episodic viral wheeze ($n = 327$), consisted of children with wheezing related only to colds (71%), mild disease (76%), and mainly normal chest x-ray results. Cluster 2, nonatopic uncontrolled wheeze ($n = 157$), was characterized by moderate to severe disease (91%), uncontrolled wheezing despite high doses of inhaled corticosteroids (55%), parents with asthma, and increased levels of ferritin. Cluster 3, atopic multiple-trigger wheeze ($n = 67$), included more children with multiple-trigger wheeze (68%) than did clusters 1 or 2; eczema (75%); a positive result from the Phadiatop Infant test (90%); increased levels of IgE, IgA, and IgG; and abnormal results from chest x-rays. In separate analysis, 1 parameter for boys (increased total level of IgE) and 2 parameters for girls (wheezing severity and increased total level of IgE) properly classified 90% of boys and 83% of girls in the appropriate cluster. Significant associations were found between overcrowding, molds and cockroaches at home, and atopic multiple-trigger wheeze and between day-care attendance and nonatopic uncontrolled wheeze in other parts. **Conclusion:** We identified different phenotypes of recurrent wheezing in young children by using cluster analysis with usual variables. These phenotypes require confirmation in longer, follow-up studies. (*J Allergy Clin Immunol* 2012;■■■:■■■-■■■.)

Key words: Risk factors, allergy, ICS, preschool children

Abbreviations used

BMI: Body mass index
EVW: Episodic viral wheeze
GINA: Global Initiative for Asthma
ICS: Inhaled corticosteroids
MTW: Multiple-trigger wheeze

Wheezing in young children is frequent and most often related to viral infection—mainly with rhinovirus or syncytial respiratory virus. Although not all children who wheeze at an early age develop asthma later in life, some wheezing infants develop early-onset asthma, which has a poor prognosis.¹ It is important to identify children at risk for early-onset asthma, to select the best therapeutic strategies, and reduce disease progression.

The Tucson Children's Respiratory Study investigated the natural history of wheezing from birth to 6 years.² Within this follow-up period, 3 phenotypes of wheezing were identified, retrospectively: early transient, late-onset, and persistent. Children with persistent wheezing have impaired lung function at age 6 years, although their lung function at earlier ages was comparable to that of children who never wheeze. This phenotype is associated with a more severe disease³ that is difficult to define. The European Respiratory Society Task Force on Preschool Wheeze recommended⁴ that preschool children with wheezing disorders^{5,6} be classified as having episodic (viral) wheeze (EVW) or multiple-trigger wheeze (MTW).⁴

However, to assign patients to these groups, an asthma classification system should be developed to distinguish among patients who present with similar clinical and biological characteristics. One approach to do so applies unsupervised statistical methods to a population that has a wide distribution of related symptoms; this approach has led to a statistical technique for identifying different phenotypes of diseases.

Using longitudinal latent class analysis, 5 wheezing phenotypes have recently been identified that were comparable in 2 large birth cohorts. These phenotypes were defined according to the age of onset and persistence during childhood: persistent wheeze (6.6%), intermediate-onset wheeze (2.6%), transient early wheeze (15.2%), prolonged early wheeze (10.1%), and late-onset wheeze (5.1%).⁷ By applying the analytical method to 2632 infants from the PARIS birth cohort during the first year of life, 2 phenotypes were defined on the basis of respiratory symptoms: a group with chronic cough (more likely to be allergic) and a group with dyspnea (more likely to be severe), compared with healthy infants.⁸

From ^athe Centre de l'Asthme et des Allergies and ^bService de Biochimie et Biologie Moléculaire, Groupe Hospitalier Trousseau-La Roche Guyon, University Paris 06; and ^cthe Laboratoire de Statistiques Théoriques et Appliquées, UPMC.

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Corresponding author: Jocelyne Just, PhD, MD, Centre de l'Asthme et des Allergies, Groupe Hospitalier Trousseau-La Roche Guyon, 26, Avenue du Docteur Arnold Netter, 75012 Paris, France. E-mail: jocelyne.just@trs.aphp.fr.

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We attempted to define a severe wheezing phenotype that is observed during the first years of life by using an unsupervised statistical approach to analyze a large population of infants with recurrent wheezing. The children were managed in a tertiary care setting and had a large distribution of disease severity. Our analysis included factors that triggered wheezing and inflammatory markers.

METHODS

Patients were part of the 3-year (2008-2010) Trousseau Asthma Program of the Centre de l'Asthme et des Allergies at the Hôpital Trousseau in Paris; the study included children with recurrent wheezing. All the children had been referred to the center by a primary care physician for persistent, recurrent wheezing; 67% were outpatients from Paris and the surrounding area, and the remaining 33% were from all regions of France. Data were collected from the children by using questionnaires and clinical examinations, following a standardized protocol, and stored in a computer database.

Inclusion criteria for the study were age younger than 36 months, active asthma (defined as a history of recurrent wheeze—more than 3 episodes of reversible bronchial obstruction documented within the previous 6 months),⁴ absence of other chronic obstructive pulmonary diseases (congenital or acquired), and without exacerbation or acute respiratory illness.

Parameters of cross-sectional data

Health outcomes were determined from a standardized questionnaire. Data were collected on children's sex, body mass index (BMI) (weight/length², measured and converted into *z* score),⁹ and maternal and paternal history of asthma. History of allergic rhinitis, eczema, and food allergy was assessed by using questions from the International Study of Asthma and Allergies in Childhood.¹⁰ Parents were asked to list factors that activated wheezing. Infants were classified as having either EVW (wheezing only during colds and remaining asymptomatic between episodes) or MTW (wheezing during colds but symptomatic between episodes, with wheezing activated by factors such as house dust, grass, pets, tobacco smoke, exercise, or cold air).

Asthma was defined as mild persistent, moderate persistent, or severe persistent according to the Global Initiative for Asthma (GINA) 2005.¹¹ All enrolled children had been on a fixed dose of inhaled corticosteroids (ICS) for at least 6 months and were compliant with their prescribed asthma treatment. High doses of ICS were defined as 250 µg or more of fluticasone (or its equivalent) per day.⁶ Asthma control was assessed according to GINA 2009.¹² Parents were also asked to report any severe exacerbations (at least 1 hospitalization).

Environmental factors

We collected data on exclusive maternal breast-feeding beyond 3 months and the extent of contact between the child and the public. This was assessed on the basis of day care attendance during the first year of life, habitation density (categorized as ≤15 m² and >15 m² per household member), and number of siblings (categorized into <3 or ≥3 siblings). We also included data on exposure to tobacco smoke (based on smokers in the home, including mother, father, or other adult household members) and automobile pollution (residence near a busy road—a standardized question from the International Study of Asthma and Allergies in Childhood). Potential sources of biologic allergens evaluated included molds (visible molds and moldy smell), furred pets (cats, dogs, and rodents), and cockroaches in the home.

Biologic markers

Nonspecific markers of inflammation were measured in peripheral blood. These included total leukocyte and neutrophil counts, expressed in absolute rate per mm³ (cell counting by automated Sysmex; Roissy, France), serum levels of IgG, IgA, and IgM (Immunoturbidimetry technique; PLC Modular, Meylan, France), and levels of ferritin and α-1-antitrypsin (Immunoturbidimetric assays; PLC Modular). Depending on the distribution of each parameter among the total population, the parameters were included as percentiles in the cluster analysis.

Biological markers of atopy were measured in peripheral blood. These included IgEs for specific inhaled and food allergens (ImmunoCAP Phadiatop Infant; Uppsala, Sweden), less-specific markers such as blood eosinophilia (cell counting by automated Sysmex; France), and total IgE (measured by ImmunoCAP). Thresholds were used to define increased levels: increased blood eosinophilia was defined as a concentration of 470 eosinophils/mm³ or more and increased total IgE as a concentration of 45 kU/L or more.¹³

Chest x-ray

Chest x-rays were classified as normal, bronchial syndrome and/or pulmonary distension, or alveolar syndrome and/or atelectasis.

Variable reduction and data transformation

The initial database included 42 variables selected from a large spectrum of routine assessments of asthmatic children. A reduction was required to perform the cluster analysis. Irrelevant variables were excluded from the final analysis (variables that did not contribute to the clinical evaluation of asthma, such as those related to neonatal assessment). Variables that were clinically redundant were excluded or reduced to avoid bias in the cluster analysis (such as absolute number of leukocytes along with absolute numbers of neutrophils or eosinophils). We retained a selection of variables that reflected physiologic parameters (eg, weight and height were replaced by BMI *z* score). For control of wheezing, a composite variable was used. Four classes were defined: controlled or uncontrolled (or partially controlled), with or without high doses of ICS. Quantitative variables (such as nonspecific biological inflammatory markers) were transformed into qualitative variables by using the 75° percentile threshold (see Table E1 in this article's Online Repository at www.jacionline.org). Environmental parameters were crossed with the clusters. Subjects were required to have all 20 variables included in the cluster analysis: demographic data (sex, BMI), variables previously reported to affect disease severity (severity, control, hospitalization for exacerbation, triggers), atopic features, and parameters with important physiologic measures (inflammatory markers, chest x-ray results).

Statistical analysis

Statistical analysis was performed with R version 2.12.0 (<http://www.r-project.org>). A hierarchical bottom-up clustering method was used to perform an unsupervised classification of the population.¹⁴ This agglomerative approach begins with each subject as a separate cluster and merges them into successively larger clusters. Ward's linkage (sample merged into larger clusters to minimize the within-cluster sum of squares) and the Euclidian distance were considered (R package, cluster). The number of clusters was evaluated from the dendrogram provided by the method. Before hierarchical clustering analysis, a dissimilarity matrix needs to be evaluated; all variables are then standardized to give them the same weight in the analysis. Gower's standardization was applied for this.¹⁴ The cluster stability was evaluated by altering the initial sample. To determine differences between clusters, analysis of variance and the Kruskal-Wallis test were used for continuous variables; χ^2 analysis and Fisher exact test were used for categorical variables.

In a second step, a decision tree was constructed by using the classification and regression trees method¹⁵ to predict the value of the cluster label based on the 20 variables used for the cluster analysis (R package, Rpart). The tree was pruned to minimize the cross-validated error. The Random Forest method,¹⁶ which consists of many decision trees, was also used (R package, Random Forest). This method, which is based on resampling techniques (bagging), is more stable and can be used to determine which variables are important in the classification.

Ethics

Parents of each child provided written informed consent before the questionnaire was distributed. Because all procedures were the standard of care for patients at the study center, the protocol was endorsed by the Institutional Review Board of the Medical Ethics Committee on Research of the Hospital Saint Antoine by the direct procedure. With respect to the

TABLE I. Clinical features of children according to cluster analysis in the entire population (N = 551)

	Total cohort (N = 551)	Cluster 1: mild EVW (n = 327)	Cluster 2: nonatopic uncontrolled wheeze (n = 157)	Cluster 3: atopic MTW (n = 67)	P value*
Health outcomes					
Sex (% male)	70	74	57	79	<.001
BMI (kg/m ²) z score	−0.05	−0.07	−0.11	0.19	.410
Allergic comorbidities					
Eczema	31	25	27	75	<.001
Allergic rhinitis	28	18	36	57	<.001
Food allergy	11	9	5	36	<.001
Maternal or paternal asthma (%)	32	26	46	30	.002
Asthma severity†					
Mild	53	76	9	42	<.001
Moderate	36	15	75	48	
Severe	11	9	16	10	
Controlled without high doses of ICS	20	30	5	7	<.001
Uncontrolled with high doses of ICS‡	28	16	55	27	
≥1 Hospitalization for asthma exacerbation	8	7	8	12	.440
Factors that induce wheezing					
EVW	59	71	45	32	<.001
MTW	41	29	55	68	

All values given as percentages, except BMI. Boldfaced text indicates statistical significance.

*Analysis of variance or χ^2 test when conditions allowed, Kruskal-Wallis or Fisher exact test otherwise.

†According to GINA.

‡Uncontrolled: no control or partially controlled. High doses of ICS were defined as 250 μ g or more of fluticasone equivalents per day.

confidentiality of patient records, data handling for the study was authorized by the Commission Nationale d'Informatique et Libertés.

RESULTS

Description of the population

We studied 551 consecutive pediatric outpatients with recurrent wheezing. There were 384 boys (69.7%), and the average age of participants was 19 ± 8.8 months. Most children had uncontrolled or partially controlled wheezing and were receiving ICS (51% were receiving high doses and 29% were receiving low doses).

Asthma phenotypes, based on cluster analysis

We used the cluster approach to generate a dendrogram that revealed 3 clusters of children with shared phenotypes (see Fig E1 in this article's Online Repository at www.jacionline.org). These clusters were identified by using data on sex, BMI, parental asthma, allergic rhinitis, eczema, food allergy, EVW versus MTW, eosinophilia, neutrophilia, levels of immunoglobulins (IgG, IgA, IgM, and IgE), levels of ferritin and $\alpha 1$ antitrypsin, results from the Phadiatop Infant test, wheezing severity (according to GINA), control by ICS, hospitalizations for severe exacerbation, and chest x-rays (Tables I and II; Fig 1).

Cluster 1. Cluster 1 was the largest group, comprising 327 children, 19 ± 8.8 months old (Tables I and II; Fig 2). This cluster had the greatest number of children with wheezing only during colds (EVW, 71% of cases) and mild severity, based on GINA classification (76%). In this group, recurrent wheezing was more frequently controlled without high doses of ICS than in the other clusters (30% compared with 5% and 7% in clusters 2 and 3, respectively). This group had the highest proportion of patients with normal chest x-rays (69%). We called this phenotype mild EVW.

Cluster 2. Cluster 2 comprised 157 children, 17 ± 8.2 months (Tables I and II; Fig 2). In this cluster, disease severity was moderate to severe (91%) and more frequently uncontrolled, despite high doses of ICS (55%). This group had a higher percentage of patients with MTW (55%) and a significantly higher proportion of parental asthma (46%) than did other clusters. The cluster also had higher proportions of patients with increased levels of ferritin (28%) and abnormal chest x-rays (bronchial syndrome and/or pulmonary distension in 44% of cases) compared with the other clusters. We called this phenotype nonatopic uncontrolled wheeze.

Cluster 3. Cluster 3 comprised 67 children, 23 ± 8.9 months old (Tables I and II; Fig 2); it included more boys than did the other clusters (79% vs 57% in cluster 2 and 74% in cluster 1). This cluster had a significantly greater percentage of children with MTW (68%) than did the other groups, indicating the increased severity of recurrent wheezing. Cluster 3 also had the highest percentages of children with atopy; the proportion with eczema was 75%, with allergic rhinitis was 57%, and with food allergy was 36%. The group had the greatest percentage of children with biologic markers of atopy; 90% had a positive result from the Phadiatop Infant test, 89% had increased levels of total IgE, and 43% had increased blood levels of eosinophils. This cluster also had the highest percentages of patients with increased levels of the nonspecific inflammatory markers IgA (48%) and IgG (37%) and with alveolar syndrome and/or atelectasis, based on chest x-rays (11%). This phenotype was called atopic MTW.

Analysis based on sex

We conducted separate cluster analyses for girls and boys. The dendrogram revealed 2 clusters of boys that corresponded to the atopic MTW and mild EVW phenotypes (see Fig E2 in this article's Online Repository at www.jacionline.org) and 3 clusters of

TABLE II. Test results of children according to cluster analysis in the entire population (N = 551)

	Total cohort (N = 551)	Cluster 1: mild EVW (n = 327)	Cluster 2: nonatopic uncontrolled wheeze (n = 157)	Cluster 3: atopic MTW (n = 67)	P value*
Nonspecific markers of inflammation†					
Blood neutrophils	25	27	23	18	.550
IgG	25	27	16	37	<.001
IgA	24	23	17	48	<.001
IgM	25	25	25	22	.390
α-1 Antitrypsin	24	23	25	28	.060
Ferritin	23	20	28	25	.007
Atopic markers					
Positive Phadiatop Infant test results	21	14	5	90	<.001
Total IgE ≥45 kU/L	31	29	10	89	<.001
Blood eosinophils ≥470/mm ³	17	12	15	43	<.001
Chest x-ray results					
Bronchial syndrome and/or pulmonary distension	31	26	44	27	<.001
Alveolar syndrome and/or atelectasis	5	5	3	11	

All values given as percentages. Boldfaced text indicates statistical significance.

*Analysis of variance or χ^2 test when conditions allowed, Kruskal-Wallis or Fisher exact test otherwise.

†Equal or above the 75th percentile.

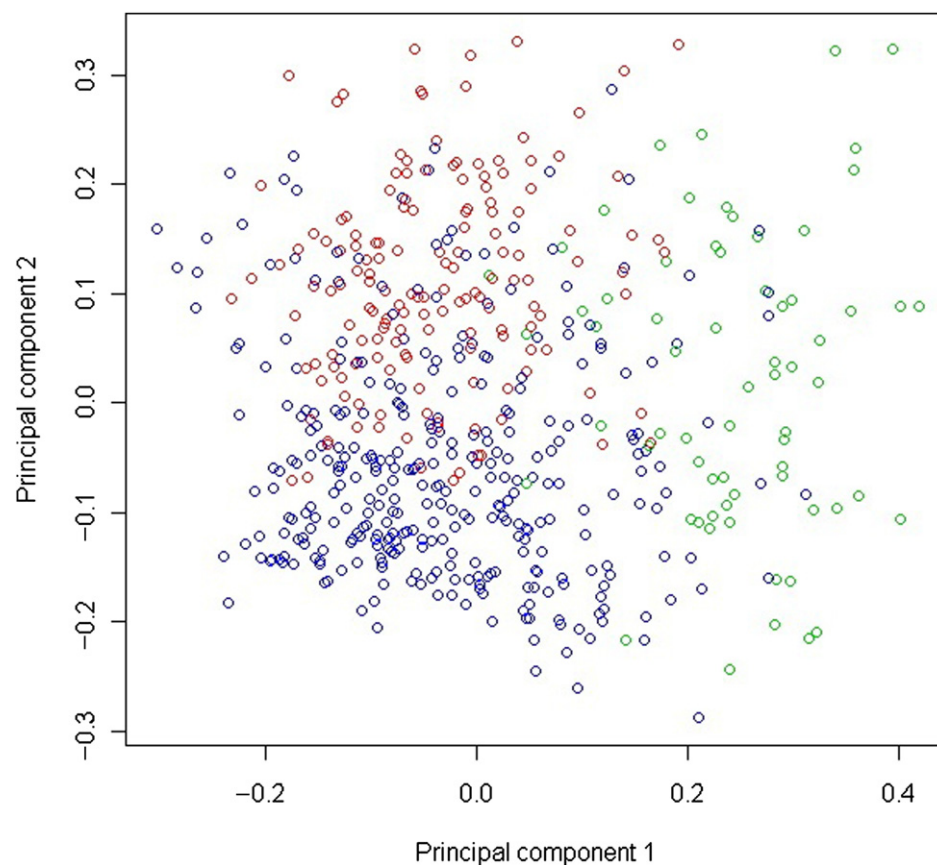


FIG 1. Scatter plot of the first 2 principal components, which display the 3 clusters identified with Ward's method (N = 551). The 2 principal components obtained captured 9.2% and 7.8% of the total variance. Each point represents a single subject. The plot depicts clustering and clear separation of children with mild EVW (n = 327) (blue circles), nonatopic uncontrolled wheeze (n = 157) (red circles), and atopic MTW (n = 67) (green circles).

girls that matched the phenotypes of wheezing described for the general population (see Fig E3 in this article's Online Repository at www.jacionline.org). The clusters were stable, because the

results persisted over time even though the statistical significance of some variables decreased slightly (see Tables E2-E5 in this article's Online Repository at www.jacionline.org).

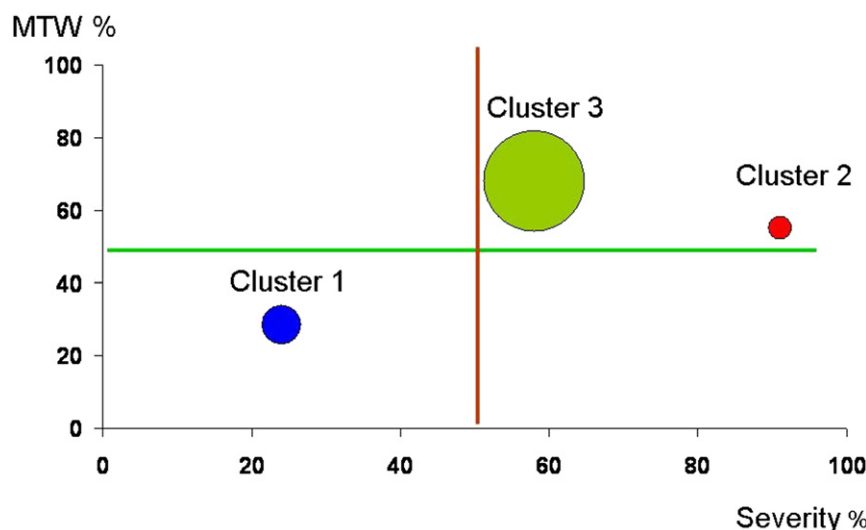


FIG 2. The 3 clusters according to the percentage of multiple-trigger wheeze, severity, and atopy (N = 551). *MTW* is defined as wheezing during colds and with other triggers such as house dust, grass, pets, tobacco smoke, exercise, or cold air. *Severity* is defined as the percentage of subjects with moderate to severe asthma, according to GINA classification. The *circled area* represents the percentage of subjects with atopy, based on positive results from the Phadiatop Infant test (≥ 0.35 U/mL).

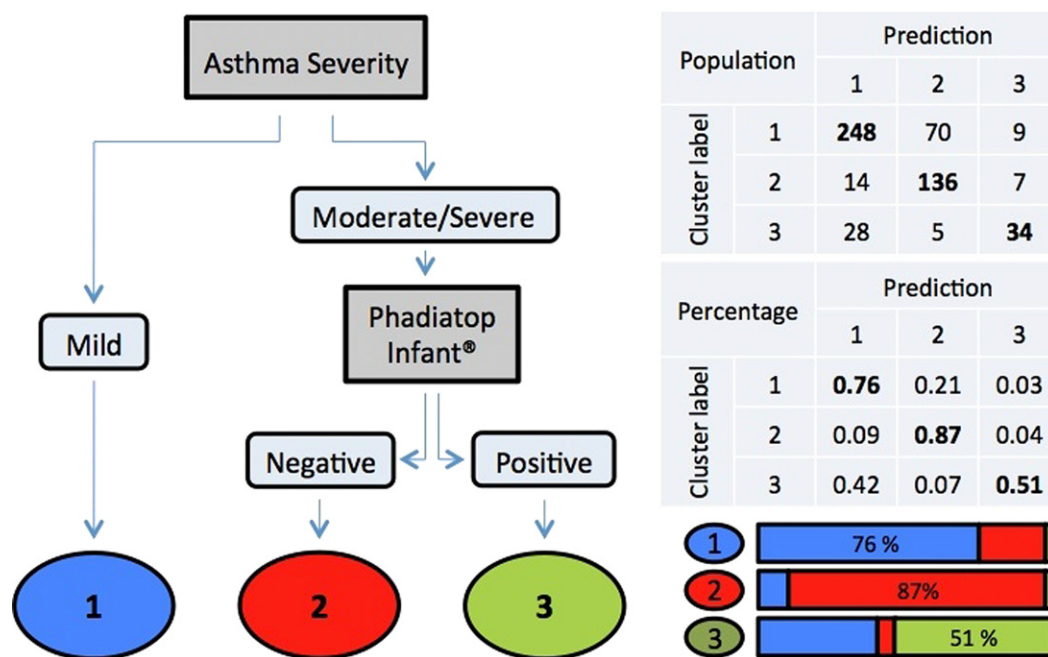


FIG 3. Classification tree for entire study population, based on 2 variables, that is, asthma severity according to GINA classification and Phadiatop Infant test. Subjects were assigned to 3 clusters that ranged from milder recurrent wheeze (cluster 1) to more severe disease (clusters 2 and 3); 76% of the subjects were assigned to the appropriate cluster. Tree performance values are given.

Classification tree and random forest

In a tree with only 2 variables—asthma severity and results from the Phadiatop Infant test—76% of the subjects were assigned to the appropriate cluster (Fig 3). These 2 variables also had the highest random forest score of importance, supporting the stability of this tree. Performing data analysis in 2 separate populations according to sex, a single parameter (increased total level of IgE) properly classified 90% of the boys (Fig 4) and 2 parameters (wheezing severity and increased total level of IgE) classified 83% of the girls in the appropriate cluster (Fig 5).

The non-overfitted prediction errors obtained with the cross-validation procedure were the same for the 3 analyses. We have therefore identified a simple method for phenotyping asthma subclasses, based on a few clinical variables.

Association of respiratory phenotypes with risk factors

Associations between phenotypes and environmental factors are reported in Table III. The presence of molds and cockroaches

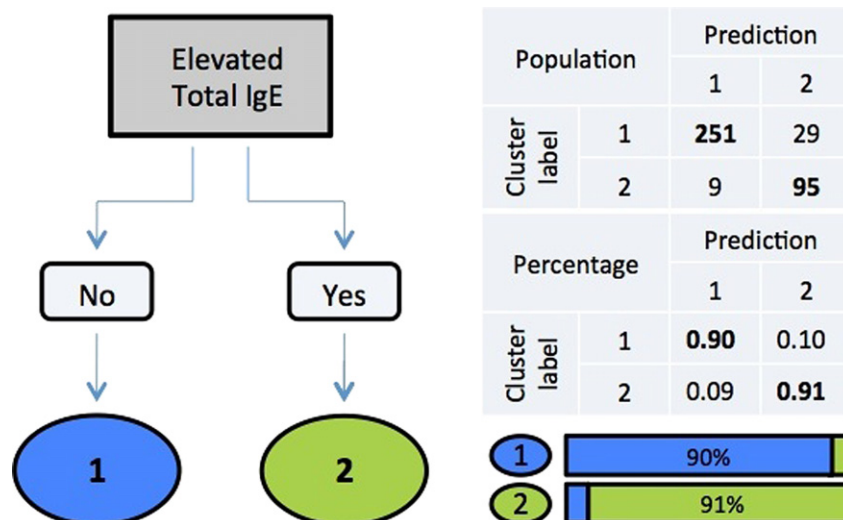


FIG 4. Classification tree for boys based on a single variable, that is, elevated total IgE as a concentration of 45 kU/L or more.¹³ Subjects were assigned to 2 clusters: milder recurrent wheeze (cluster 2) or atopic disease (cluster 1); 90% of the subjects were assigned to the appropriate cluster. Tree performance values are given.

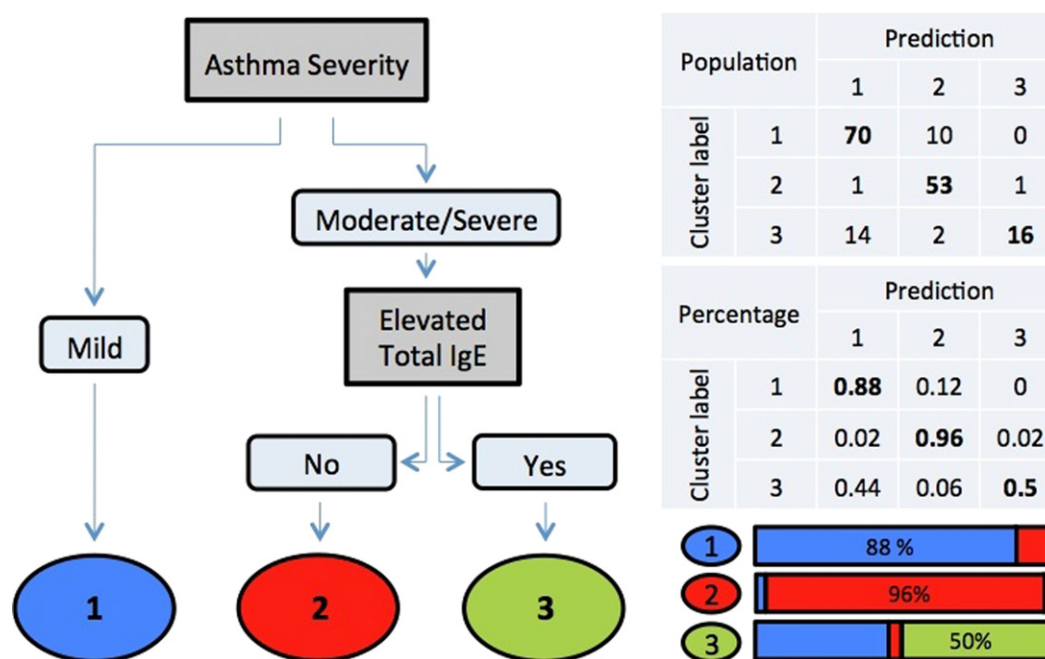


FIG 5. Classification tree for girls, based on 2 variables, that is, asthma severity according to GINA classification and elevated total IgE as a concentration of 45 kU/L or more.¹³ Subjects were assigned to the 3 clusters, ranging from milder recurrent wheeze (cluster 1) to more severe disease (clusters 2 and 3); 83% of the subjects are assigned to the appropriate cluster. Tree performances are given.

and overcrowding at home were significantly associated with cluster 3 (atopic MTW) ($P = .021$, $P = .018$, and $P = .004$, respectively). Conversely, day-care attendance in the home was significantly associated with cluster 2 (nonatopic uncontrolled wheeze) ($P = .035$).

DISCUSSION

We identified 2 novel phenotypes of severe wheezing, which we called atopic MTW and nonatopic uncontrolled wheeze,

compared with a phenotype of milder, recurrent wheeze, which we called mild EVW.

Previously reported phenotypes of wheezing preschool children, but not infants, were classified as EVW and MTW, based on expert recommendations for treatment.⁴ Sonnappa et al¹⁷ associated MTW with significant abnormalities in pulmonary function, independent of atopy or wheezing status. Our analysis of wheezing in infants incorporated data on induction by single or multiple factors, atopy, and other markers of disease severity (including therapeutic control of wheezing).

TABLE III. Environmental features of children according to cluster analysis in the entire population (N = 551)

	Total cohort (N = 551)	Cluster 1: mild EVW (n = 327)	Cluster 2: nonatopic uncontrolled wheeze (n = 157)	Cluster 3: atopic MTW (n = 67)	P value*
Breast-feeding ≥ 3 mo	43	43	36	58	.015
Tobacco smoke exposure	28	25	32	36	.130
Day-care attendance	46	45	56	36	.035
Mold exposure	19	18	16	31	.021
Cockroach exposure	8	6	7	17	.018
Overcrowding†	29	28	23	46	.004
Furred pet exposure	19	18	24	17	.270

All values given as percentages. Boldfaced text indicates statistical significance.

*Analysis of variance or χ^2 test when conditions allowed, Kruskal-Wallis or Fisher exact test otherwise.

†Overcrowding ≤ 15 m² per inhabitant.

Atopic MTW

Atopic MTW is associated with severe wheezing; 68% of these patients have MTW, as well as clinical and biological features of allergy. The association we observed between allergy (defined as allergic comorbidities) and the severity of wheezing is supported by previous findings. In a prospective study of German patients,¹⁸ eczema that was associated with loss-of-function mutations in fil-aggrin and sensitization to food allergens increased the risk for severe asthma, characterized by significant decreases in pulmonary function at puberty. The first manifestation of allergic rhinitis can occur in preschool children,¹⁹ which seems to predict the development of asthma between the ages of 5 and 13 years in the same cohort.²⁰ Allergic comorbidities, such as food allergy, were more frequently observed in patients with severe acute asthma that required the use of emergency services or intensive care.^{21,22}

Atopic MTW is also characterized by nonspecific inflammation; these patients are more likely to have increased levels of IgG and IgA and abnormal results from chest x-rays. Little is known about the association between atopic asthma and nonspecific markers of inflammation. Moreover, independently of IgE sensitization, mold and dampness can promote the growth of bacteria and house-dust mites, which release compounds that irritate the respiratory system and cause nonspecific inflammation.²³ Mold increases the risk of recurrent wheezing, especially among allergic infants (5-fold in food or aeroallergen-sensitized infants and nearly 2-fold in nonsensitized infants).²⁴⁻²⁶ The association between this atopic phenotype and cockroach exposure is consistent with other surveys, which associated exposure to cockroaches with wheezing among 2-year-olds—especially among children with a maternal history of asthma.²⁷ Environmental factors (exposure to mold and cockroaches and overcrowding) could be indicators of low socioeconomic status and risk factors for asthma among inner-city children.^{28,29}

The effect of breast-feeding on the development of asthma is controversial. Sears et al³⁰ found that the prevalence of atopy and asthma increased with the duration of breast-feeding, independently of the family history of asthma and allergic rhinitis. Kuyucu et al³¹ associated the duration of breast-feeding and the presence of atopy in children with the atopic status of mothers; the risk of atopy in children decreased only when mothers without atopy breast-fed their children long periods of time. The association observed between the duration of breast-feeding and atopic MTW in our study population could be explained by the confounding effect of socioeconomic status and atopic disease in mothers, rather than the duration of breast-feeding.

Separate analyses of girls and boys increased the percentages of children correctly assigned in appropriate clusters. Among boys, only 1 severe cluster was described: atopic MTW compared with Mild EVW. This might be related to associations between male sex and allergic diseases.³²⁻³⁵

Nonatopic uncontrolled wheeze

The phenotype of nonatopic uncontrolled wheeze was observed most commonly in children with MTW; it is characterized by a significantly higher frequency of uncontrolled wheezing, despite treatment with high doses of ICS, significantly higher blood levels of ferritin, and day-care attendance. Nonspecific inflammatory markers were assessed because results from the Trousseau Asthma Program cohort showed that they could discriminate among asthma phenotypes.³⁶ This cluster had a significant number of children with parents who had a history of asthma.

Attending day care could increase the exposure of young children to infectious agents. Microbial exposures might, somewhat paradoxically, protect against atopic asthma but be risk factors for nonatopic asthma. Airway inflammation from the microbiota induces uncontrolled asthma³⁷ and corticosteroid resistance.³⁸ Some researchers³⁹ have proposed that persistent respiratory infections contribute to the development of intrinsic asthma. In our study, this severe cluster was observed only in girls. Rackemann and Mallory⁴⁰ first described intrinsic asthma with a female predominance over 50 years ago, though the etiology is still not understood. Another study reported the effects of parental history of asthma on the development of childhood asthma, independently of atopic status.⁴¹

Strengths and limitations

One strength of this study is that it objectively measured atopy and infectious inflammation in a large sample (more than 500) of children with recurrent wheezing. There are only a few birth cohorts from which multiple biologic markers of atopy have been measured within the first years of life,⁴² and we do not know of any that have collected data on nonspecific inflammation. During infancy, the development of atopy varies with the maturity of the immune system. It therefore appeared relevant to study atopy according to different standards, including allergen-specific sensitization to foods and inhaled allergens included in the Phadiatop Infant test, blood levels of eosinophilia, and total serum levels of IgE.^{13,19} Another strength of our study was that we assessed

a large cohort of severely ill infants by using unsupervised cluster analysis, which allowed us to identify different phenotypes without *a priori* assignment and to test the stability of the clusters.

One limitation of our study was that all the patients came from a single center. However, Paris and its surrounding areas are large and contain more than 10 million people of diverse origins. Our results should, however, be confirmed in a general population that has not received therapy for asthma.

Conclusions

In a large study of infants with recurrent wheezing, we identified 2 severe phenotypes of MTW: atopic MTW, associated with allergenic factors, and nonatopic uncontrolled wheeze, associated with infectious factors. These results indicate that simple methods can be used to assign phenotypes to infants with recurrent wheezing, based on usual variables. However, these phenotypes need to be confirmed in studies that follow these patients for longer time periods.

Clinical implications: We described 2 phenotypes of infants with severe wheezing. Boys more frequently had atopic, multiple-trigger wheezing with allergenic environments, whereas girls had nonatopic uncontrolled wheezing, associated with infectious agents.

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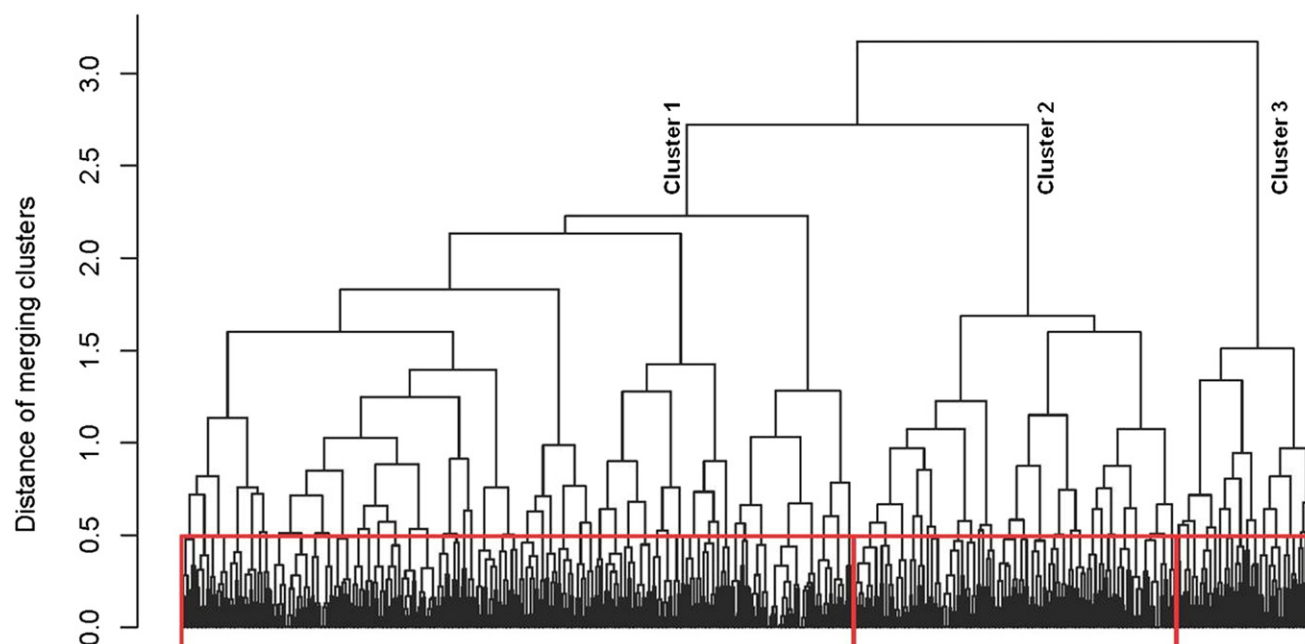


FIG E1. Dendrogram, for the entire population ($N = 551$), obtained with a hierarchical bottom-up clustering method. Three clusters were apparent. This agglomerative approach begins with each subject as a separate cluster and merges them into successively larger clusters. By Ward's linkage, samples were merged into larger clusters to minimize the within-cluster sum of squares.

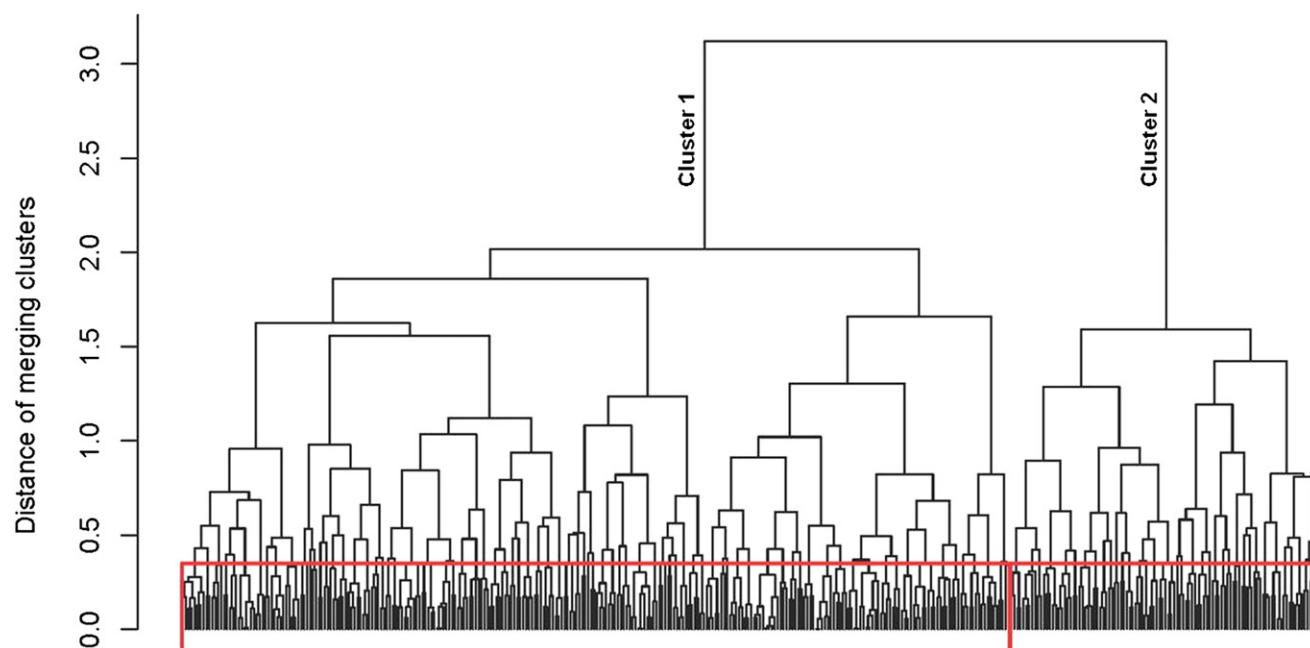


FIG E2. Dendrogram, for boys ($n = 384$), obtained with a hierarchical bottom-up clustering method. Two clusters were apparent. This agglomerative approach begins with each subject as a separate cluster and merges them into successively larger clusters. By Ward's linkage, samples were merged into larger clusters to minimize the within-cluster sum of squares.

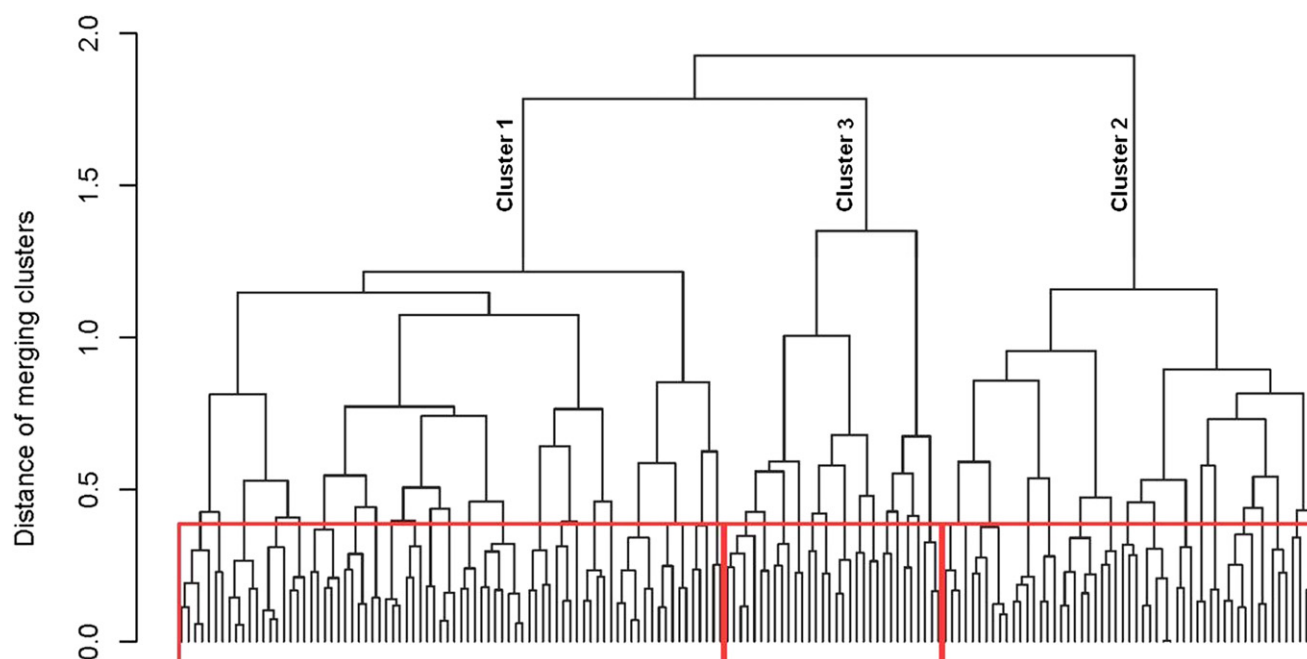


FIG E3. Dendrogram, for girls ($n = 167$), obtained with a hierarchical bottom-up clustering method. Three clusters were apparent. This agglomerative approach begins with each subject as a separate cluster and merges them into successively larger clusters. By Ward's linkage, samples were merged into larger clusters to minimize the within-cluster sum of squares.

TABLE E1. Quantitative values of nonspecific inflammatory markers for the entire population (N = 551)

	25th percentile	75th percentile
Blood neutrophils (cells/mm ³)	2410	4880
IgG (g/L)	5.28	8.25
IgA (g/L)	0.26	0.64
IgM (g/L)	0.61	1.1
α-1 Antitrypsin (g/L)	1.17	1.44
Ferritine (μg/L)	22	47

The nonspecific inflammatory markers included in cluster analysis were equal or above the 75th percentile. Boldfaced text indicates statistical significance.

TABLE E2. Clinical features of children according to cluster analysis in boys (n = 384)

	Total cohort (n = 384)	Cluster 1: mild EVW (n = 280)	Cluster 2: atopic MTW (n = 104)	P value*
Health outcomes				
BMI (kg/m ²) z score	0.01	0.00	0.02	.920
Allergic comorbidities				
Eczema	29	24	43	<.001
Allergic rhinitis	26	23	31	.190
Food allergy	11	8	18	.009
Maternal or paternal asthma	19	33	29	.640
Asthma severity†				
Mild	53	54	53	.990
Moderate	34	34	35	
Severe	13	13	12	
Controlled without high dose of ICS	55	18	49	.330
Uncontrolled with high dose of ICS‡	29	31	23	
≥1 Hospitalization for asthma exacerbation	9	7	13	.140
Triggers				
EVW	59	64	47	.005
MTW	41	36	53	

All the variables are expressed in percentages, except BMI. Boldfaced text indicates statistical significance.

*Analysis of variance or χ^2 test when conditions were respected, Kruskal-Wallis or Fisher exact test otherwise.

†According to GINA.

‡Uncontrolled: no control or partially controlled.

TABLE E3. Paraclinical features of children according to cluster analysis in boys (n = 384)

	Total cohort (n = 384)	Cluster 1: mild EVW (n = 280)	Cluster 2: atopic MTW (n = 104)	P value*
Nonspecific inflammatory markers†				
Blood neutrophils	25	30	15	<.001
IgG	21	19	30	<.001
IgA	25	18	46	<.001
IgM	20	21	20	.11
α-1 Antitrypsin	21	23	26	.12
Ferritin	22	25	19	.45
Atopic markers				
Positive Phadiatop Infant	20	8	57	<.001
Total IgE ≥45 kU/L	32			
Blood eosinophils ≥470/mm ³	16	12	30	<.001
Chest x-ray				
Bronchial syndrome and/or pulmonary distension	32	37	17	<.001
Alveolar syndrome and/or atelectasis	5	4	8	

All the variables are expressed in percentages. Boldfaced text indicates statistical significance.

*Analysis of variance or χ^2 test when conditions were respected, Kruskal-Wallis or Fisher exact test otherwise.

†Equal or above the 75th percentile.

TABLE E4. Clinical features of children according to cluster analysis in girls (n = 167)

	Total cohort (n = 167)	Cluster 1: mild EVW (n = 80)	Cluster 2: nonatopic uncontrolled wheeze (n = 55)	Cluster 3: atopic MTW (n = 32)	P value*
Health outcomes					
BMI (kg/m ²) z score	0.13	−0.26	0.03	−0.42	.650
Allergic comorbidities					
Eczema	35	39	26	41	.140
Allergic rhinitis	33	26	36	44	.116
Food allergy	12	9	11	22	.150
Maternal or paternal asthma	19	38	33	11	.086
Asthma severity†					
Mild	51	88	2	44	<.001
Moderate	40	10	75	53	
Severe	10	3	24	3	
Controlled without high dose of ICS	22	35	4	19	<.001
Uncontrolled with high dose of ICS‡	29	6	65	22	
≥1 Hospitalization for asthma exacerbation	7	1	13	9	.024
Triggers					
EVW	59	77	40	47	<.001
MTW	41	23	60	53	

All the variables are expressed in percentages, except BMI. Boldfaced text indicates statistical significance.

*Analysis of variance or χ^2 test when conditions were respected, Kruskal-Wallis or Fisher exact test otherwise.

†According to GINA.

‡Uncontrolled: no control or partially controlled.

TABLE E5. Paraclinical features of children according to cluster analysis in girls (n = 167)

	Total cohort (n = 167)	Cluster 1: mild EVW (n = 80)	Cluster 3: nonatopic uncontrolled wheeze (n = 32)	Cluster 2: atopic MTW (n = 55)	P value*
Nonspecific inflammatory markers†					
Blood neutrophils	23	28	20	16	.550
IgG	32	33	19	55	.010
IgA	21	14	13	55	<.001
IgM	32	19	38	61	<.001
α-1 Antitrypsin	21	17	23	44	.048
Ferritin	20	21	23	18	.927
Atopic markers					
Positive Phadiatop Infant	19	15	6	53	<.001
Total IgE ≥45 kU/L	25	14	2	90	<.001
Blood eosinophils ≥470/mm ³	17	15	15	26	.360
Chest x-ray					
Bronchial syndrome and/or pulmonary distension	30	20	40	38	.014
Alveolar syndrome and/or atelectasis	6	3	9	9	

All the variables are expressed in percentages. Boldfaced text indicates statistical significance.

*Analysis of variance or χ^2 test when conditions were respected, Kruskal-Wallis or Fisher exact test otherwise.

†Equal or above the 75th percentile.