

Cluster Analysis Identifies 3 Phenotypes within Allergic Asthma



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What is already known about this topic? Unbiased approaches have shown that patients with asthma can be clustered in different phenotypes, some of them allergic. No study has phenotyped asthmatic allergic patients.

What does this article add to our knowledge? We propose for the first time new allergic asthma phenotypes based on a family history of allergy, age of onset, and severity of asthma.

How does this study impact current management guidelines? Careful classification of patients can contribute to understanding mechanisms in allergic asthma, explaining discrepancies in gene association studies, and improving the clinical approach of these patients.

BACKGROUND: Asthma is a heterogeneous chronic disease with different clinical expressions and responses to treatment. In recent years, several unbiased approaches based on clinical, physiological, and molecular features have described several phenotypes of asthma. Some phenotypes are allergic, but little is known about whether these phenotypes can be further subdivided.

OBJECTIVE: We aimed to phenotype patients with allergic asthma using an unbiased approach based on multivariate classification techniques (unsupervised hierarchical cluster analysis).

METHODS: From a total of 54 variables of 225 patients with well-characterized allergic asthma diagnosed following American Thoracic Society (ATS) recommendation, positive skin prick test to aeroallergens, and concordant symptoms, we finally selected 19 variables by multiple correspondence analyses. Then a cluster analysis was performed.

RESULTS: Three groups were identified. Cluster 1 was constituted by patients with intermittent or mild persistent

asthma, without family antecedents of atopy, asthma, or rhinitis. This group showed the lowest total IgE levels. Cluster 2 was constituted by patients with mild asthma with a family history of atopy, asthma, or rhinitis. Total IgE levels were intermediate. Cluster 3 included patients with moderate or severe persistent asthma that needed treatment with corticosteroids and long-acting β -agonists. This group showed the highest total IgE levels.

CONCLUSIONS: We identified 3 phenotypes of allergic asthma in our population. Furthermore, we described 2 phenotypes of mild atopic asthma mainly differentiated by a family history of allergy. © 2017 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2018;6:955-61)

Key words: Allergic asthma; Cluster analysis; Phenotypes; Inheritance

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testimony from Novartis, Boehringer-Ingelheim, GlaxoSmithKline, and AstraZeneca; has received lecture fees from Novartis, Boehringer-Ingelheim, Esteve, Almirall, Chiesi, Menarini, GSK, Takeda, Pfizer, Ferrer, AstraZeneca, and Stallergenes. I. Dávila has received research and travel support from ISCIII; has received consultancy fees from AstraZeneca, Sanofi, and Novartis; has received research support from ThermoFisher and Merck; has received lecture fees from AstraZeneca, GlaxoSmithKline, ALK, Stallergenes, and Chiesi. The rest of the authors declare that they have no relevant conflicts of interest.

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Abbreviations used

BMI- Body mass index
 FENO- Fractional exhaled nitric oxide
 GEMA- Spanish Guideline on the Management of Asthma
 GINA- Global Initiative for Asthma
 LABA- Long-acting β -agonists
 SARP- Severe Asthma Registry Program

Asthma is a chronic inflammatory disease affecting more than 300 millions of people worldwide and causing approximately 250,000 deaths annually.¹ It is considered a heterogeneous disease with different levels of severity, age of onset, type of inflammation, response to treatments, and triggers. This heterogeneity has been recognized in guidelines, such as Global Initiative for Asthma (GINA) (www.ginasthma.org) and Spanish Guideline on the Management of Asthma (GEMA) 4.0.² Accordingly, the interest on phenotyping asthma has almost exponentially increased during the last decade.³ A phenotype can be considered the external manifestation of an individual's underlying genetics on interaction with environment. Asthma was first phenotyped by Rackemann,^{4,5} who classified the disease as allergic or extrinsic and nonallergic or intrinsic. Initially, phenotypic classifications of asthma were performed using biased approaches, that is, based on the observation of common characteristics of patients to describe different types of asthma. Thus, these classifications were based on clinical or physiological features, triggers, or the type of inflammation.⁶ In recent years, unbiased approaches, that is, based mainly on statistical methods, such as clusters or latent class analyses have been introduced. Although some bias is introduced during the process of variable selection,⁷ several important studies have been published,⁸⁻¹⁰ followed by others^{11,12}; recently, molecular phenotypes have emerged.¹³ In 2017 GINA guidelines¹⁴ (www.ginasthma.org), the following phenotypes are considered: allergic asthma, nonallergic asthma, late onset asthma, asthma with fixed airway limitation, and asthma with obesity. Although not all the studies describe the same phenotypes, the allergic phenotype seems to be present in all studies. Nevertheless, the allergic phenotype is a broad phenotype with different degrees of severity.⁸ In addition, atopy is present across different phenotypes. In the present study, we aimed to further phenotype allergic asthma.

METHODS

A database was elaborated from data obtained from the clinical history of patients evaluated in real life conditions in the Department of Allergy of the University Hospital of Salamanca (Spain) from 2003 to 2014. The review of clinical histories was developed from 2014 to 2016. After reviewing 700 histories, 225 patients fulfilling the inclusion criteria were selected. Data were obtained from the clinical history and electronic records. All data introduced in the database were anonymized, thus avoiding the possibility of patient identification. The study was approved by the Ethics Committee of Clinical Investigation of the hospital (2014/11/131A). All patients included in our registry were informed that their data would be anonymously used for scientific purposes.

Subjects

All patients had been evaluated by an allergist and had signed an informed written consent to be studied in the outpatient clinic. The inclusion criteria were: (1) age over 14 years; (2) asthma diagnosed

following GEMA 2009,¹⁵ including either a positive bronchodilator-methacholine test; (3) at least 1 positive skin prick test from a locally adapted battery of common aeroallergens performed following European Academy of Allergy and Clinical Immunology recommendations¹⁶ or specific serum IgE; and (4) symptoms and signs of asthma concordant with allergen exposure. The exclusion criteria were: (1) negative skin prick tests; (2) other pulmonary diseases; and (3) patients who did not accept the tests routinely performed at our patient clinic.

Globally, 54 variables were recorded from the clinical history (see Table E1 in this article's Online Repository at www.jaci-inpractice.org). To be properly managed, variables were selected before performing the cluster analysis. Thus, those variables lacking a significant number of individuals (with missing data), being redundant or irrelevant for the purposes of this study, or with low contributions in the solution of multiple correspondence analysis,¹⁷ were rejected (Fig 1). The finally selected variables, on the basis of their considered contribution to the asthma phenotype, were age, sex, body mass index (BMI), family history of atopy (defined as a first-degree antecedent with atopic dermatitis, asthma, or allergic rhinitis), family history of asthma, family history of rhinoconjunctivitis, age of onset of asthma, age of onset of rhinitis, atopic dermatitis, allergic rhinoconjunctivitis, smoking status, lung function, fractional exhaled nitric oxide (FENO), eosinophil peripheral blood count, serum total IgE levels, severity of asthma according to GEMA 2009,¹⁵ use of inhaled steroids, use of long-acting bronchodilators, and use of oral corticosteroids.

Statistical analysis

To perform the analysis of the collected multidimensional information 2 multivariate techniques were performed. First, multiple correspondence analysis,¹⁷ a nonlinear reduction technique for the reduction of dimensionality, was used in 2 ways: on the one hand, to statistically analyze all the variables initially collected in this study and measured in different scales (sociocultural and demographic variables, clinical and functional characteristics of asthma, family history, etc.), to determine among them the most informative variables; and, on the other hand, to obtain a low-dimensional solution (latent numeric variables) on which to perform additional analysis. Secondly, on the numerical solution previously obtained we carried out a cluster analysis (exploratory data analysis), a multivariate technique used to obtain classification systems. The purpose was to sort patients into groups or clusters, so the degree of association/similarity among members of the same cluster was stronger than the degree of association/similarity among members of different clusters. This statistical procedure allows discovering structures in the data that *a priori* are not usually obvious and thus contributing to the formal definition of a phenotype for a set of given objects. All statistical analyses were performed using the SPSS version 22 (IBM SPSS Statistics for Windows, Version 22.0; IBM Corp., Armonk, NY).

RESULTS

The final analysis included 225 subjects who had complete data for the 19 selected variables. The demographics of the entire cohort are presented in Table I. Briefly, 213 patients had both asthma and rhinitis and 12 had only asthma. In the global sample (Table I), there was a predominance of females (57.3%); median age was 39 years and mean BMI was 24.49 kg/m². Mean IgE was 362.07 kU/L; mean of peripheral eosinophils was 309.78/ μ L; and mean fractional nitric oxide was 48.82 ppb.

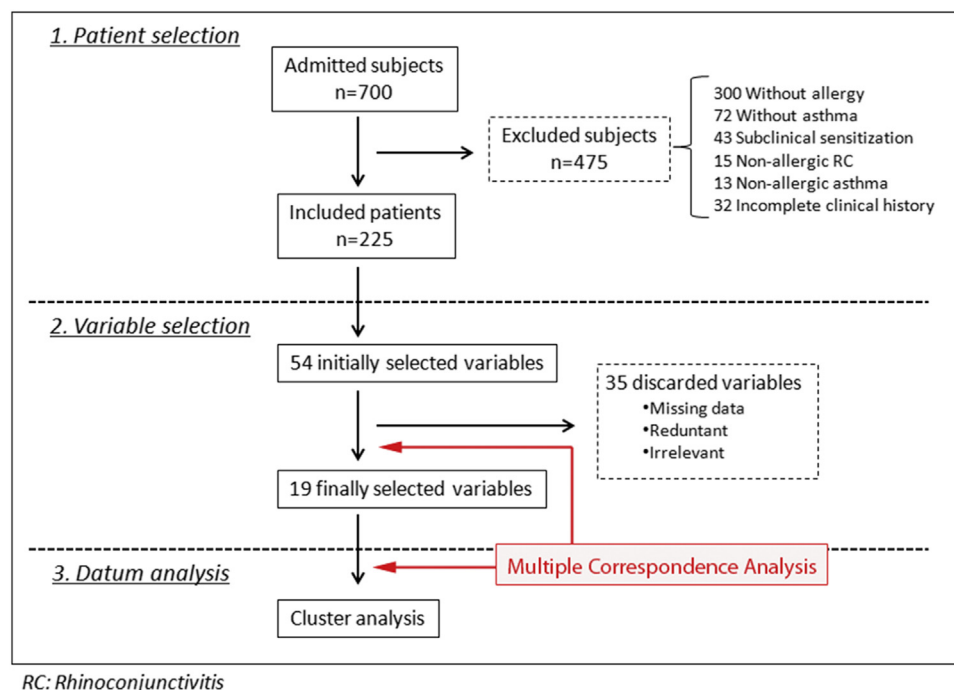


FIGURE 1. Flow chart of working process.

Multidimensional analysis

From the final data matrix (225 patients by 19 variables), a k-means cluster analysis was performed to identify relatively homogeneous groups of patients from the information provided by the solution of the multiple correspondence analysis,¹⁷ performed from the original 19 variables (this was the solution that collected 63.2% of the total information contained in the original data matrix). As a method of clustering we selected the Ward method (or minimum variance method), a hierarchical agglomerative procedure in which a dendrogram plot was generated (Fig 2), for appreciation of the number of likely clusters within the patients. The final adopted solution was constituted by 3 clusters (Fig 3), cluster 1 being the greatest, including 59.1% of patients, and cluster 3 the smallest, with only 12% of patients (Table II). The values for each variable of the 3 clusters are presented in Tables II and III. The characterization of each cluster was tested by comparing the variables of the agglomerative methodology, and thus the analysis of one-way variance was used for age and BMI variables and on the log transformed continuous variables,¹⁹ that is, total IgE, peripheral blood eosinophil counts, and FENO (therefore, parametric outcomes), and the χ^2 test for the proportions.

Cluster 1. Cluster 1 was the largest cluster, including 133 patients (59.11%) with a mean age of 29.56 years and predominance of females (56.4%). Most patients had intermittent (58.6%) or mild persistent asthma (11.3%). Lung function was normal in most of the patients. Accordingly, only 31.6% required inhaled corticosteroids and only 30.8% required long-acting β -agonists (LABA). This group was best characterized by the observation that almost all of these patients lacked a family history of atopy (93.9%), asthma (97.7%), or rhinitis (98.5%). In most of them, asthma was initiated before they were 18 years old. The mean total IgE level was the lowest of the 3 clusters

($P = .077$). Mean FENO levels were 44.31 ppb and mean peripheral eosinophil levels were 304.02/ μ L.

Cluster 2. This cluster included 65 patients (28.88%). Mean age was 29 years and there was a female predominance (60%). In general, asthma severity was mild, with most of the patients having intermittent asthma (58.5%). Accordingly, lung function was normal in 83.6% of patients and most of the patients did not require inhaled corticosteroids (80%) or LABA (83.1%). Asthma was initiated before 18 years in most of them (59.8%). The more striking feature of this cluster is that all patients (100%) had a family history of atopy. In addition, most of them also had a family history of asthma (64.1%) and rhinitis (68.8%). The mean total IgE level was 468.93 kU/L. Mean FENO and peripheral eosinophil levels were similar to those of cluster 1.

Cluster 3. This was the smallest cluster (27 patients, 12%), but they had more severe asthma. There was a predominance of females (55.6%). Thus, 81.5% had moderate or severe persistent asthma, and most of them were receiving inhaled corticosteroids (88.9%). In addition, 85.2% of patients were receiving LABA. Lung function tests showed an obstructive pattern in most individuals (92.6%). Slightly more than half of the patients had a family history of atopy. Interestingly, in most of them (65.4%), asthma had begun in adulthood. The mean total IgE level was the greatest of the 3 clusters ($P = .077$) as well as mean FENO levels (although no significant differences were found between clusters).

DISCUSSION

In this study, we have evaluated only patients with allergic asthma. Using an unsupervised hierarchical clustering method, we identified 3 clusters: 2 of them (clusters 1 and 2) were constituted by patients mainly suffering from mild asthma and the third one (cluster 3) by patients mainly suffering from moderate-to-severe

TABLE 1. Demographics characteristics of the entire cohort (n = 225)

Variable	Mean (median)	SD (IQR)
Age (y)	39.56 (31)	18.54 (17)
BMI (kg/m ²)	24.94 (24.49)	4.30 (4.95)
Total IgE (kU/L)	362.07 (199.05)	393 (399.03)
Blood eosinophils/ μ L	309.78 (265)	193.18 (238)
FENO (ppb)	48.82 (34.5)	42.08 (46)
Variable	Categories	Percentage
Sex	Female	57.3
Current smoking status	Yes	12.9
Onset of asthma <18 years old	Yes	56.7
First-degree family history of atopy	Yes	38.9
First-degree family history of asthma	Yes	23.9
First-degree family history of rhinitis	Yes	22.6
Total serum IgE levels (kU/L)	<100	26
	101-1000	64.9
	>1000	9.1
FEV ₁ (% of predicted)	<60	2.2
	60-80	19.8
	>80	78
FEV ₁ /FVC (%)	<70	13.7
	>70	86.3
FEF ₂₅₋₇₅ (% of predicted)	<65	32.6
	>65	67.4
Use of inhaled corticosteroids	Yes	32.1
Use of LABA	Yes	33.3
Severity of asthma (GEMA 2009)	Intermittent	52
	Mild persistent	13.3
	Moderate persistent	30.3
	Severe persistent	4.4
Step of asthma treatment	Step 1	64.9
	Step 2	1.8
	Step 3	18.7
	Step 4	12.4
	Step 5	1.3
	Step 6	0.9

Spirometric reference values of Casan et al.¹⁸

BMI, Body mass index; FEF₂₅₋₇₅, forced expiratory flow at 25% to 75% of forced vital capacity; FENO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in 1 s; FVC, forced volume vital capacity; GEMA, Spanish Guideline on the Management of Asthma; IQR, interquartile range; LABA, long-acting β -agonists; SD, standard deviation.

asthma. The main differences were based on the age of onset of asthma, severity, and family antecedents of atopy. The most striking feature is that the 2 mild allergic asthma clusters are differentiated by the family antecedents of atopy, which were lacking in cluster 1 and were present in all patients of cluster 2.

Asthma is a complex disease and it has been suggested to be more a syndrome than a disease.²⁰ Allergic asthma is the most common phenotype in the general population of patients with asthma.²¹ In recent years, there has been an increasing interest in the phenotyping of asthma.³ As previously stated, several studies based on cluster analysis for the identification of clinical asthma phenotypes have been published in the past few years.⁸⁻¹⁰ These

studies have found several different phenotypes in which the percentage of atopy varied significantly among them,^{8,9} although in a recent cluster analysis from the Severe Asthma Registry Program (SARP) based on both sputum inflammatory cells and clinical characteristics, atopy was present at a similar frequency in all the 4 groups.²² So in this study, we decided to analyze only patients with allergic asthma instead of a global population of asthmatics.

The clusters we found seem to partially correspond to already described clusters. Thus, clusters 1 and 2 are in agreement with mild allergic asthma clusters, such as cluster 1 of the primary care population of Moore et al,⁹ cluster 1 of Haldar et al,⁸ or cluster A of Moore et al.²² The more severe cluster 3 could correspond to cluster 4 of the SARP study,⁹ or to cluster 1 of the secondary care study of Haldar et al.⁸ In any case, it has to be taken into account that in the previously described studies atopy is distributed across all phenotypes. In this sense, with our study we have tried to avoid the possible confusion of atopy, as all patients had atopic asthma.

What is new in our study is the further subdivision of mild atopic asthmatics into 2 subphenotypes: cluster 1 without family antecedents of atopy (mild atopic asthma without a family history of atopy) and cluster 2 with family antecedents of atopy (mild atopic asthma with a family history of atopy). From a clinical point of view, these 2 groups are indistinguishable as age, severity, age of onset, lung function, FENO, and peripheral blood eosinophils are nondiscriminating variables. In this sense, both of them seem to have been integrated in the above-mentioned milder phenotypes of the previously described studies. The only difference could be in total IgE levels (although differences were not statistically significant, there was a clear trend, $P = .077$), which were more elevated in cluster 2, which is precisely the cluster with a positive family history of atopy. It is clear that allergic sensitization is the result of both genetic and environmental interactions. Thus, although in both clusters IgE is a key etiopathogenic factor, as evident by positive specific IgE or skin prick testing and symptoms on exposure to the allergen, it is tempting to speculate that in cluster 1 the effect of environmental influences could be more evident than that of genetic influences, whereas in cluster 2 it could be the other way around. In this sense, associations between total serum IgE and polymorphisms of different genes have been described.²³⁻²⁵ This could be reflected in the higher levels of total serum IgE observed in cluster 2, in which genetic influences are purportedly stronger. In the case of cluster 3, in which a family history of atopy is present in less than a half of patients, the higher serum IgE levels could be related to a higher severity.²⁶

To understand differences between clusters 1 and 2, we could speculate with different scenarios that need not necessarily exclude each other. First, the so-called “missing heritability” has been observed for most complex diseases.^{27,28} The absence of a family history of asthma observed in cluster 1 could be related to the heritability of asthma. In a recent meta-analysis, asthma heritability was estimated in 0.54,²⁹ although increased figures have also been reported.³⁰ This fact is in agreement with our results, and it could be related to the fact that different phenotypes could have been included in the different studies of heritability. It could also contribute to explaining inconsistencies and lack of replication of gene association studies of asthma in which phenotypes of patients did not take into account a family history of the disease.³¹ In this sense, Bønnelykke and Ober³¹ suggested that the extreme clinical heterogeneity of asthma is barely

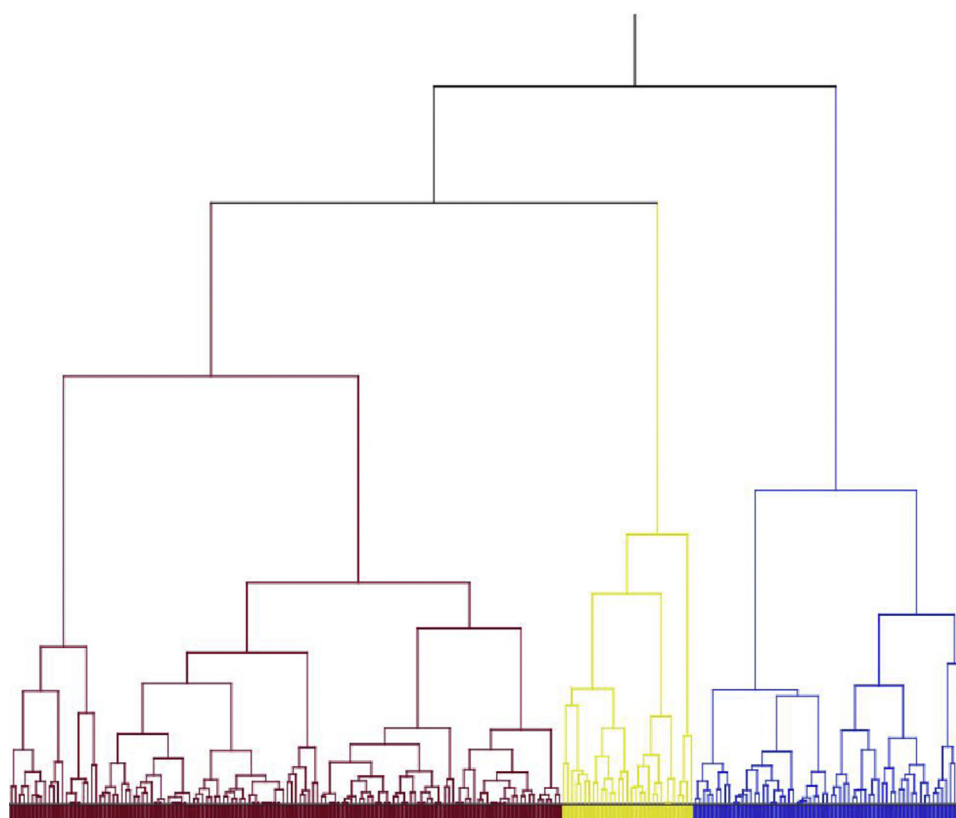


FIGURE 2. Dendrogram (hierarchical cluster analysis, Ward method). Cluster 1 is represented in red, cluster 2 is represented in yellow, and cluster 3 is represented in blue.

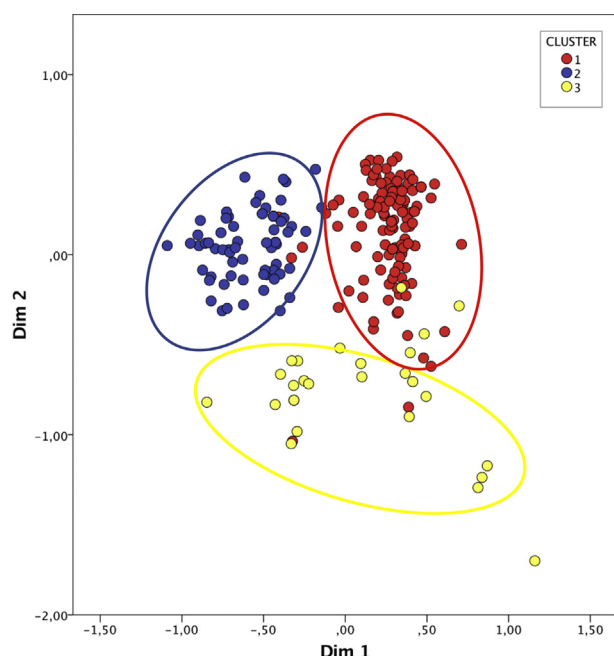


FIGURE 3. Representation, on the first factorial plane obtained in the multiple correspondence analysis solution, of the 3 clusters obtained after hierarchical clustering analysis (Ward method).

considered in the large samples required for genome-wide association studies. On the other hand, differences between clusters 1 and 2 could also be related to different inheritance patterns that could be involved in regulatory mechanisms of gene expression. Finally, a different interaction between asthma and environmental factors could be accounted for. Recently, it has been described that changes in the prevalence of environmental factors can modify the penetrance of asthma susceptibility genotypes that manifest the effects through gene-environmental interactions.³¹ In this sense, epigenetics could bring light to this question.³² In any case, this could raise the question that putative mechanisms could be underlying the differences observed between both clusters, which could open new insight regarding endotypes, considered as subtypes of diseases defined by different functional or pathological mechanisms.³³

Another remarkable result is based on differences with cluster 3. This cluster differentiates it from clusters 1 and 2 in the severity of asthma and in the age of onset of asthma. It has previously been shown that the age of onset of asthma is an important factor in asthma phenotypes.⁷ This also could be related to different genetic factors with variable expression or with a different interaction with environmental factors, which points again to probable epigenetic factors modulating the development of the disease in these patients.

In conclusion, we have identified 3 phenotypes in allergic asthma: mild atopic asthma without a family history of atopy, mild atopic asthma with a family history of atopy, and moderate-severe atopic asthma. These phenotypes are

TABLE II. Statistics of quantitative variables of each cluster

Variable	Cluster 1 N = 133 (59.1%)	Cluster 2 N = 65 (28.9%)	Cluster 3 N = 27 (12%)	P value ANOVA (log values)*
Age (y)				
Mean (SD)	29.56 (11.88)	29 (11.88)	45.30 (19.40)	<.001
BMI				
Mean (SD)	24.80 (4.89)	24.09 (3.69)	24.59 (3.55)	.730
Total IgE				
GM†	188.04	266.32	271.97	.077
Mean (SD)	334.50 (411.68)	468.93 (478.50)	550.54 (965.52)	
Blood eosinophils/μL				
GM†	219.40	227.78	246.03	.825
Mean (SD)	304.02 (269.41)	295.04 (220.05)	292.82 (159.59)	
FENO (ppb)				
GM†	32.96	37.38	41.87	.521
Mean (SD)	44.31 (38.65)	49.88 (42.21)	60.67 (63.50)	

ANOVA, Analysis of one-way variance; BMI, body mass index; FENO, fractional exhaled nitric oxide; SD, standard deviation.

*Log values were applied to total IgE, blood eosinophils, and FENO.

†GM denotes geometric mean.

TABLE III. Statistics of qualitative characteristics of each cluster

Variable	Categories	Cluster 1 N = 133	Cluster 2 N = 65	Cluster 3 N = 27	P value
Sex	Male	43.6%	40.0%	44.4%	.873
Smoking status	Yes	12%	18.5%	3.7%	.141
Onset of asthma <18 years old	Yes	59.7%	59.7%	34.6%	.053
First-degree family history of atopy	Yes	6.1%	100%	53.8%	<.001
First-degree family history of asthma	Yes	2.3%	64.1%	34.6%	<.001
First-degree family history of rhinitis	Yes	1.5%	68.8%	15.4%	<.001
Total serum IgE levels (kU/L)	<100	31.5	18.8	16	.055
	101-1000	61.5	65.6	80	
	>1000	6.9	15.6	4	
FEV ₁ (percentage of predicted value)	<60	—	—	18.5%	<.001
	60-80	17.2%	16.4%	40.7%	
	>80	82.8%	83.6%	40.7%	
FEV ₁ /FVC	<70	4.3%	—	92.6%	<.001
	>70	95.7%	100%	7.4%	
FEF ₂₅₋₇₅ (percentage of predicted value)	<65	25.9%	21.3%	92.6%	<.001
	>65	74.1%	78.7%	7.4%	
Use of inhaled corticosteroids	Yes	31.6%	20%	88.9%	<.001
Use of LABA	Yes	30.8%	16.9%	85.2%	<.001
Severity of asthma (GEMA 2009) ¹⁵	Intermittent	58.6%	58.5%	3.7%	<.001
	Mild persistent	11.3%	16.9%	14.8%	
	Moderate persistent	28.6%	23.1%	55.6%	
	Severe persistent	1.5%	1.5%	25.9%	
Step of asthma treatment	Step 1	68.4%	80%	11.1%	<.001
	Step 2	0.8%	3.1%	3.7%	
	Step 3	18.8%	12.3%	33.3%	
	Step 4	10.5%	3.1%	44.4%	
	Step 5	1.5%	—	3.7%	
	Step 6	—	1.5%	3.7%	

Spirometric reference values of Casan et al.¹⁸FEF₂₅₋₇₅, Forced expiratory flow at 25% to 75% of forced vital capacity; FEV₁, forced expiratory volume in 1 s; FVC, forced volume vital capacity; GEMA, Spanish Guideline on the Management of Asthma; LABA, long-acting β-agonists.

differentiated mainly by age of onset, family history of allergy, and severity. Further studies to define the pathogenetic, prognostic and therapeutic responsiveness implications of these phenotypes are warranted.

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TABLE E1. Initially selected variables

Gender	First-degree family history of atopy
Age	First-degree family history of asthma
Skin tests	First-degree family history of allergic rhinitis
Food allergy	Atopy in the mother
Drug allergy	Asthma in the mother
Smoking status	Nonsteroidal drug hypersensitivity
Asthma severity	Nasal polyps
Age of onset of asthma	Degree of nasal polyps
Asthma duration	Allergen immunotherapy
Allergic rhinitis	Treatment step (GEMA 2009)
Classification of allergic asthma	Asthma control (GEMA 2009)
Age of onset of allergic rhinitis	Emergency visit episodes in the last year
Atopic dermatitis	Hospital admissions
Body mass index	Intensive care unit admissions
Forced vital capacity (L)	Inhaled corticosteroids
Forced vital capacity (% of predicted value)	Inhaled corticosteroids (dose)
Forced expiratory volume in 1 s (FEV ₁) (L)	Long-acting bronchodilators
FEV ₁ (% of predicted value)	Long-acting bronchodilators (dose)
FEV ₁ /FVC	Oral steroids
Forced expiratory flow (FEF ₂₅₋₇₅) (L/s)	Oral steroids (dose)
FEF ₂₅₋₇₅ (% of predicted value)	Number of cycles of oral steroids
Bronchodilator test	Theophyllines
Fraction of exhaled nitric oxide	Theophyllines (dose)
Methacholine test	Anticholinergics
Peripheral blood eosinophil count	Anticholinergics (dose)
Peripheral blood eosinophil count (percentage)	Omalizumab
Total serum IgE	Omalizumab (dose)

FVC, Forced volume vital capacity; GEMA, Spanish Guideline on the Management of Asthma.

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