

ORIGINAL ARTICLE

Airways Disease

Contribution of repeated infections in asthma persistence from preschool to school age: Design and characteristics of the PreDicta cohort

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Abstract

Background: The PreDicta cohort was designed to prospectively evaluate wheeze/asthma persistence in preschoolers in association with viral/microbial exposures and immunological responses. We present the cohort design and demographic/disease characteristics and evaluate unsupervised and predefined phenotypic subgroups at inclusion.

Methods: PreDicta is a 2-year prospective study conducted in five European regions, including children 4-6 years with a diagnosis of asthma as cases and healthy age-matched controls. At baseline, detailed information on demographics, asthma and allergy-related disease activity, exposures, and lifestyle were recorded. Lung function, airway inflammation, and immune responses were also assessed. Power analysis confirmed that the cohort is adequate to answer the initial hypothesis.

Results: A total of 167 asthmatic children (102 males) and 66 healthy controls (30 males) were included. Groups were homogeneous in respect to most baseline characteristics, with the exception of male gender in cases (61%) and exposure to tobacco

Abbreviations: FEF₅₀, forced expiratory flow in the mid (50%) of FVC; FeNO, fractional exhaled nitric oxide; FEV_{0.5}, forced expiratory volume in 0.5 seconds; FEV₁, forced expiratory volume in the first second; FVC, forced vital capacity; PEF, peak expiratory flow; Ppb, parts per billion; RV, rhinovirus; SPT, skin prick test.

smoke. Comorbidities and number and duration of infections were significantly higher in asthmatics than controls. 55.7% of asthmatic children had at least one positive skin prick test to aeroallergens (controls: 33.3%, $P = .002$). Spirometric and exhaled nitric oxide values were within normal limits; only baseline $FEV_{0.5}$ and FEV_1 reversibility values were significantly different between groups. Viral infections were the most common triggers (89.2%) independent of severity, control, or atopy; however, overlapping phenotypes were also common. Severity and control clustered together in an unsupervised analysis, separating moderate from mild disease.

Conclusions: The PreDicta cohort presented no differences in non-asthma related measures; however, it is well balanced regarding key phenotypic characteristics representative of “preschool asthma”.

KEYWORDS

asthma, PreDicta, preschoolers, viral infections

1 | INTRODUCTION

Although the diagnosis of asthma in infants and preschoolers can be challenging,¹ the vast majority of asthma cases in older children and adults originate from recurrent wheezers in the first year of life.² Interactions of both genetic and environmental factors, including viral and microbial infections, exposure to pollutants, and more, have been implicated in asthma inception.^{3,4} However, the factors implicated in disease persistence and progression may differ from those that initiate or exacerbate it. Allergen-specific sensitization, especially early in life, is considered an important risk factor for asthma persistence, although its role in asthma initiation is still debated.⁵

Viral respiratory infections, particularly those caused by rhinoviruses (RVs), might play a pivotal role in asthma development⁶ and have been well established as the most frequent triggers of mild common colds and acute asthma exacerbations⁷; nevertheless, their role in asthma/respiratory allergy persistence is less clear. Previously, we have shown that RV infection promotes airway remodeling through induction of growth factors,^{8,9} while repeated infection-triggered asthma episodes result in prolonged bronchial hyperresponsiveness, more so in atopic asthmatics, potentially increasing the likelihood of asthma persistence and/or the transition from “recurrent wheeze” to “persistent asthma”.¹⁰ Moreover, a significant causal role of allergic sensitization in the severity of virus-induced wheezing and subsequent asthma development has been proposed.¹¹ It is well known that viruses exhibit a complex interaction with the human immune system, depending on various factors such as age, atopy, infectious agent, severity, and site of the infection.¹²

Patterns of remission, relapse, and new disease development at any age¹³ suggest that the natural history and progression of “wheeze” to asthma may not be deterministic, that is, “decided” at an early stage by either a genetic pattern or an environmental exposure, but rather indeterministic (probabilistic or stochastic), that is, continuously developing in relation to

ongoing—not necessarily predictable—exposures. The overall hypothesis of PreDicta, a 7th Framework Programme for Research and Technological Development (FP7) European Commission-funded project, is that repeated, infection-mediated events may reprogram immune responses toward a chronic inflammation pattern, thus leading to asthma persistence. Within the PreDicta project, the PreDicta pediatric longitudinal cohort was designed to prospectively evaluate wheeze/asthma persistence in preschool to school-age children, in association with ongoing microbial exposures and concurrent immune responses.

Herein, we present the cohort design, describe the demographic and disease characteristics of the population, and evaluate unsupervised and predefined phenotypic subgroups at inclusion. We conclude that the PreDicta population is balanced regarding key phenotypic characteristics representative of “preschool asthma” and can thus address the study hypothesis.

2 | METHODS

2.1 | Study population

Children 4–6 years of age with an asthma diagnosis¹⁴ of mild to moderate severity according to GINA¹⁵ within the last 2 years, confirmed by a doctor of the participating study center, were invited to participate as cases. Eligibility criteria included at least 3 wheezing episodes in the preceding 12 months (1 in the last 6 months) and child's ability to perform a peak expiratory flow (PEF) maneuver. Exclusion criteria were having a history of severe/brittle asthma, being in need of more than 6 courses of oral steroids in the preceding 12 months, being on immunotherapy, having a history of a chronic respiratory disease, except allergic rhinitis, and undergoing chronic medication use. An episode of an upper respiratory infection and/or asthma exacerbation during the preceding 4 weeks prior to inclusion and baseline evaluation was also an exclusion criterion.

Healthy, age-matched children, with no reported history of asthma/wheeze except rhinitis, served as cross-sectional controls at the beginning and the end of the 2-year follow-up period. They were recruited from surgical wards undergoing a planned minor operation, while blood sampling was a part of an already scheduled diagnostic procedure.

The study was approved by all participants' institutional ethics committees, and written informed consent was obtained from parents.

2.2 | Study design

The PreDicta cohort has been conducted as a 2-year prospective study, across five major cultural and climatic regions of Europe: Greece (Athens), Germany (Erlangen), Belgium (Ghent), Poland (Lodz), and Finland (Turku). At inclusion, participants were 4-6 years old. The selected age bracket was considered appropriate for the evaluation of asthma persistence, as the disease is expected to transform (resolve or persist) within a 2-year time frame in a considerable number of patients.¹⁶ Regular follow-up was scheduled every 6 months. Patients and their parents were provided with and trained to fill in electronic diary cards implemented in a cell phone and perform peak flow measurements in a matched peak flow meter (<https://www.ixsys.eu/telemedicine-network.html>) 3 times per week. The system was set to transmit the data via Wi-Fi in a specific, secure platform.

At recruitment, a baseline questionnaire including demographic characteristics was filled in and detailed information on asthma and other allergy-related diseases' activity and severity was obtained. Information on exposures and lifestyle, including physical activity, infection history, and exposures to tobacco smoke, allergens, and pollutants, was also obtained.

Spirometry was performed following training. PEF, FVC, FEV_{0.5}, FEV₁, and FEV₅₀ were recorded before and after bronchodilation with 400 mcg of salbutamol delivered through a metered-dose inhaler with a spacer. The devices used in the centers were the same as the ones used for monitoring at home (Contec, SP10 spirometers). The ERS reference values, implemented in a spirometer software, were used for analysis.¹⁷

Airway inflammation was determined with fractional exhaled nitric oxide (FeNO) measurement in parts per billion (ppb) using the Bedfont NObreath equipment (Bedfont Ltd, UK),¹⁸ whereas bronchial hyperresponsiveness was determined with a free running test.¹⁹

Atopy was assessed with skin prick testing (SPT) to a panel of standard allergens kindly provided by Allergopharma, Germany. These included the following: normal saline, histamine (10 mg/mL), a dermatophagoides mix, cockroach, cat, alternaria, and a grass mix²⁰; birch and ambrosia were also used in Ghent, Lodz, Erlangen, and Turku, and parietaria and olive in Athens. The largest wheel diameter in mm was recorded.²¹

At baseline and at the end of the 2-year follow-up period, peripheral blood was obtained to determine antibodies for rhinoviruses, lipid mediators (resolvins, protectins, and lipoxins), atopic

sensitization by MeDALL-chip, staphylococcus-specific IgE, and vitamin D in the serum. Moreover, whole blood was used for epigenetic and transcriptomic profiling and for evaluating the cytokine responses of peripheral mononuclear cell cultures to various stimuli. Nasopharyngeal samples were obtained at baseline and during 6-month follow-up visits for the assessment of bacterial and viral colonization. All material was obtained as depicted in Table S2.

Moreover, children were assessed at clinic during a cold and/or asthma exacerbation and 4-6 weeks later at convalescence (at least on two different occasions) through a questionnaire, clinical examination, spirometry, and FeNO. Nasopharyngeal samples (for viral and bacterial detection) and peripheral blood for RV antibody determination were also obtained.

2.3 | Statistical analysis

It was calculated via a one-sided log-rank test power analysis that an overall sample size of 166 subjects (of which 99 are expected to be atopic) achieves 80% power at a 0.05 significance level to detect a difference of 20% of the patients that will remain asthmatic and prospectively evaluate the effects of infections and atopy as predictors, including an extra proportion of 20% loss to follow-up patients or patients who will not have events (e.g., exposure to viral infections).

Nominal variables are presented using absolute and relative frequencies (%), continuous variables with mean, standard deviation, median, and interquartile range (25th-75th percentile) depending on whether a variable follows the normal distribution or not. Normality tests of continuous variables were performed using the Kolmogorov-Smirnov test and histograms. A chi-squared test of independence was performed to evaluate associations between two nominal variables. The Mann-Whitney and Kruskal-Wallis tests were used to evaluate associations between continuous and nominal variables with two or more categories, respectively. A linear regression analysis with interaction term was used to compare baseline data between centers.

Statistical significance was set at $P < .05$. IBM SPSS Statistics 20.0 (SPSS Inc., Chicago, IL, USA) was used for the analyses.

2.4 | Data preprocessing

Missing values were treated with the MICE (Multivariate Imputation by Chained Equations) package in R to impute the missing values using different imputation methods for each type of data: Predictive mean matching was used for numeric variables, logistic regression for binary variables, and Bayesian multinomial logistic regression for factor variables with more than two levels.

2.5 | Data clustering

Two-step random forest clustering was used to group the patients based on the measured clinical data. In the first step, the dissimilarity measure is used to represent each subject (patient) as a point

TABLE 1 Baseline characteristics and environmental exposures in asthmatic and healthy preschoolers

Baseline characteristics	Cases N = 167	Controls N = 66	P value
Age (y) mean $\pm$ SD	5.2 $\pm$ 0.7	5.1 $\pm$ 0.8	.23
Sex (boys), n (%)	102 (61.0)	30 (45.4)	.03
Day care attendance (yes), n (%)	158 (94.6)	62 (93.9)	.84
Vigorous physical activity (times per wk)			
Once/twice, n (%)	28 (16.8)	13 (19.7)	.59
Three times or more, n (%)	85 (50.9)	36 (54.5)	
TV attendance (h/d)			
<1 h, n (%)	41 (24.6)	32 (48.5)	.002
1-3 h, n (%)	104 (62.3)	27 (40.9)	
Paternal education level (secondary), n (%)	74 (44.3)	28 (42.4)	.91
Maternal education level (secondary), n (%)	61 (36.5)	25 (37.9)	.95
Father smoker (yes), n (%)	49 (29.3)	16 (24.2)	.43
Number of cigarettes father smokes, mean (SD)	15.8 (9.5)	12.9 (9.4)	.36
Mother smoker (yes), n (%)	29 (17.4)	7 (10.6)	.19
Number of cigarettes mother smokes, mean (SD)	12 (8.59)	9.2 (7.6)	.21
Number of cigarettes smoked at home, mean (SD)	1.68 (5.35)	0.48 (2.8)	.01
Frequent exposure to traffic pollution, n (%)	32 (18.6)	12 (18.2)	.83
Mold spots or window condensation (yes), n (%)	44 (26.3)	12 (18.2)	.19
Pet ownership (yes), n (%)	70 (41.9)	22 (33.3)	.22
Cat (yes), n (%)	30 (46.2)	10 (47.6)	.82
Dog (yes), n (%)	31 (44.1)	13 (61.9)	.54
History of breastfeeding (yes), n (%)	139 (83.2)	53 (80.3)	.59
Breastfeeding months, median (CI)	4 (1-8)	4 (2-9)	.24

bold values are statistical significant.

in a two-dimensional space using multidimensional scaling.²² In the second step, the point distances are used for clustering. The clustering algorithms were hierarchical algorithms, k-means, divisive analysis (DIANA), partitioning around medoids (PAM), and clustering large application (CLARA), while the optimum number of clusters was based on the silhouette width measure using the R package clValid. Cluster analysis was performed with an R software.

3 | RESULTS

3.1 | Baseline characteristics and environmental exposures

A total of 167 cases (102 males, 61%) and 66 controls (30 males, 45%) were recruited from the five participating centers. The respective number of cases and controls per center is as follows: Athens: 37/15, Erlangen: 24/22, Ghent: 22/15, Lodz: 41/5, and Turku: 43/9. Mean age at recruitment was 5.2 $\pm$ 0.7 for the cases and 5.1 $\pm$ 0.8 for the controls. One hundred and thirty-nine asthmatic children completed the 2-year follow-up (dropout rate: 16.76%). The respective dropout rates for the centers were as follows: Athens: 13.5%, Erlangen: 25%, Ghent: 63.6%, Lodz: 2.4%, and Turku: 4.6%.

Table 1 shows demographic and exposure characteristics of the groups at inclusion. Asthmatic children included more boys, and increased hours of television attendance and a higher exposure to tobacco smoke were reported. Otherwise, the groups were homogeneous.

3.2 | Personal and family history of atopy

Comorbid allergy-associated diseases, such as allergic rhinitis, atopic dermatitis, and adverse reaction to foods and insect stings, were more frequent in the asthmatics than in the controls (Table 2). 55.7% of asthmatic children had at least one positive SPT to aeroallergens, while the respective percentage for the controls was 33.3% ($P = .002$). Children with doctor-diagnosed rhinitis were more frequently atopic compared to normal controls (66.4% as assessed by SPTs, $P < .001$). Moreover, a positive family history of asthma and rhinitis was more frequent in the asthmatic preschoolers than in the controls.

3.3 | Infection history

Reported infections during the last 12 months were generally higher and lasted longer in the asthmatic group (Table 3). The cases were also prescribed significantly more antibiotics.

3.4 | Asthma activity and symptoms in asthmatic preschoolers

Seasonal patterns of asthma activity were similar across Europe. The lowest activity was during the summer months (July and August). A sharp increase was noted in autumn, with peak incidence from September to November, remaining high during winter and dropping either gradually during spring (Ghent, Erlangen, and Turku) or sharply after April/May (Athens and Lodz) (Figure 1).

With respect to asthma activity in the 3-month period prior to inclusion, the frequency of daytime symptoms was >once/week but less than daily in 28.1% and daily in 10.2% of the asthmatic cohort, while night symptoms >2 times/mo and >once/wk were reported in 13.2% and 18.6% of the asthmatics, respectively.

TABLE 2 Reported personal and family history of allergy-related diseases in asthmatic and non-asthmatic preschoolers

Personal and family history of allergic diseases	Cases n (%) (Total = 167)	Controls n (%) (Total = 66)	P value
Current allergic rhinitis symptoms	139 (83.2)	22 (33.3)	<.001
Doctor-diagnosed allergic rhinitis (ever)	112 (67.1)	1 (1.5)	<.001
Current atopic dermatitis symptoms	57 (34.1)	3 (4.5)	<.001
Doctor-diagnosed atopic dermatitis (ever)	86 (51.5)	4 (6.1)	<.001
Adverse reaction to foods: cow's milk, egg, fish, wheat, peanut, nuts, apple, and peach (ever)	53 (31.7)	7 (10.6)	.001
Allergic reaction to wasp or beesting	21 (12.6)	1 (1.5)	.009
Allergy to latex (patients)	3 (1.8)	0 (0.0)	.27
Severe anaphylaxis (patients) ^a	6 (3.6)	0 (0.0)	.12
Any positive skin prick test to aeroallergens	93 (55.7)	22 (33.3)	.002
Father asthma (ever)	37 (22.2)	2 (3.0)	.001
Father allergic rhinitis (ever)	73 (43.7)	15 (22.7)	.003
Father atopic dermatitis (ever)	28 (16.8)	6 (9.1)	.13
Father adverse reaction to foods (ever)	25 (15.0)	9 (13.6)	.79
Mother asthma (ever)	36 (21.6)	6 (9.1)	.03
Mother allergic rhinitis (ever)	64 (38.3)	11 (16.7)	.001
Mother atopic dermatitis (ever)	34 (20.4)	8 (12.1)	.14
Mother adverse reaction to foods (ever)	31 (18.6)	8 (12.1)	.20
Allergy-associated diseases in siblings (cases = 135/controls = 53)			
Doctor-diagnosed asthma	36 (26.6)	5 (9.4)	.01
Doctor-diagnosed allergic rhinitis	38 (28.1)	2 (3.8)	<.001
Doctor-diagnosed atopic dermatitis	36 (26.7)	13 (24.5)	.76
Doctor-diagnosed food allergy	20 (14.8)	5 (9.4)	.32

^aAccording to Reference.³⁹

bold values are statistical significant.

Median numbers of wheezing/asthma episodes during 3 and 12 months prior to inclusion were 1 (IQR: 1-3) and 5 (IQR: 3-9), respectively, while no hospitalizations for asthma were recorded. With regard to asthma prophylactic treatment, 80% were on inhaled corticosteroids, 15% on long-acting β_2 -agonists (23/25 combined with inhaled steroids), and 38% on antileukotrienes (14/64, 21.8% as monotherapy).

3.5 | Lung function and airway inflammation

91% of the children were able to perform PEF and FVC maneuvers, while the percentages for FEV₁ and FEF were 80% and 68%, respectively. FeNO values were recorded in 79% of the cases. Baseline spirometric and FeNO values were within normal limits. FEV_{0.50} (pre-bronchodilation) and FEV₁ reversibility values were increased in the asthmatics compared to the controls ($P = .04$ and $.01$, respectively). Although numerical differences between the groups were observed, these were not significant (Table 4).

3.6 | Asthma phenotyping

Asthmatic children were grouped by level of severity according to GINA as follows: 30% as intermittent and 55% and 15% as mild and moderate persistent, respectively, while classification by level of control showed that 49.7% were controlled, 36.5% partly controlled, and 13.8% uncontrolled.

Trigger-based phenotypes were also assessed.¹⁴ Viral infections were the most common precipitating factor (68.7%), followed by exercise (26.5%) and exposure to relevant allergen (24.1%); nevertheless, overlapping phenotypes were also common, as shown in Figure 2.

3.7 | Between centers analysis

Overall, baseline data were homogeneous between centers; however, with respect to prophylactic treatment, regular inhaled

corticosteroid use ranged from 52% in Athens to 98% in Turku, while montelukast was mainly reported in Athens (36%) and Lodz (48%). There was also diversity among reported triggers, asthma severity, and control (Table S1).

3.8 | Clustering of clinical data

To unravel possible clusters among asthmatic patients, random forest dissimilarity distances were calculated for all patients in the dataset and were represented as points in two-dimensional scaling

TABLE 3 Reported infections 12 month prior to inclusion

	Cases (N = 169) Median (IQR)	Controls (N = 66) Median (IQR)	P value
Upper airway respiratory infections	5 (3-8)	4 (2-6)	.001
Maximum duration of upper respiratory infections	7 (7-14)	7 (5.7-10)	.14
Lower airway respiratory infections	1 (0-1.2)	0 (0.0-0.0)	<.001
Maximum duration of lower respiratory infections	7.5 (0-14)	0 (0-0)	<.001
Otitis media	0 (0-1)	0 (0-1)	.62
Maximum duration of otitis media	4.5 (0-8.5)	0 (0-7)	.06
Gastrointestinal infections	0 (0-1)	0 (0-1)	.65
Maximum duration of gastrointestinal infection	3 (0-5)	1 (0-3)	.01
Antibiotic courses	2 (1-4)	1 (0-2)	.001
Maximum duration of antibiotic courses	10 (7-10)	7 (0-8)	<.001

bold values are statistical significant.

plots. To assess which parameters were important in the distances among the points (patients), two metrics were considered as follows: the mean decrease accuracy and the mean decrease Gini (Figure S1). Silhouette width plots (Figure S2) show that the optimum number of clusters that separate the data is 2 for all tested clustering algorithms, while the two large groups (the asthmatics and the healthy controls) are visually depicted in the multidimensional plot of the random forest dissimilarity (Figure S3).

As asthmatics are well separated from controls, a new dataset that included only cases was considered. Important parameters for patient separation are shown in Figure S4. Silhouette width plots of the cases reveal that the optimum clustering algorithm and number of clusters are k-means clustering and 3 clusters, respectively (Figure S5). A multidimensional scaling plot that includes 3 clusters in the case dataset and one for the controls is depicted in Figure 3.

The unsupervised clusters reflected overall disease activity well, including severity, control, and comorbidity, with mild, moderate, and high activity groups (Table 5).

4 | DISCUSSION

The PreDicta multicenter pediatric cohort is well balanced and powered to prospectively evaluate the role of repeated infections—in addition to atopy—in asthma persistence and is representative of preschool asthmatics, as was shown by analysis of baseline and disease characteristics. The cohort size was delimited by intensive follow-up and frequent sampling. Phenotyping of asthmatic children confirmed that virus-induced asthma is the most common disease presentation in preschoolers, independent of severity, control, or atopy, although a significant proportion of children present mixed phenotypes. Measures of respiratory allergy activity, including asthma severity, control, and allergic rhinitis comorbidity, clustered together in an unsupervised analysis, separating moderate from milder disease.

Most of the demographic characteristics were equally distributed between the cases and the controls in our cohort; males

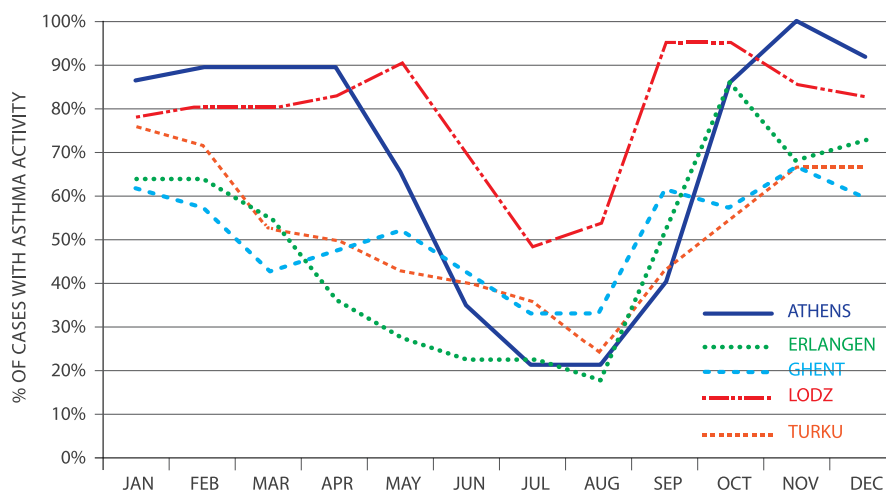


FIGURE 1 Seasonality of reported asthma symptoms in PreDicta centers across Europe. Percentage of children with asthma symptoms 12 month prior to inclusion [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 4 Spirometric and FeNO values in the cases and the controls at baseline

	Cases Median predicted Z-scores (%)	Controls Median predicted Z-scores (%)	P value
Pre-PEF (L/min) (cases = 152, controls = 57)	-1.66	0.48	.53
Post-PEF (L/min) (cases = 145, controls = 41)	-0.06	-0.02	.75
PEF reversibility (% change)	6.0 (-5.0 to 14.0)	-2.0 (-11.2 to 14.5)	.11
Pre-FVC (L) (cases = 152, controls = 55)	-0.14	0.08	.44
Post-FVC (L) (cases = 143, controls = 39)	-0.15	0.05	.38
FVC reversibility (% change)	1.0 (-5.0 to 8.0)	1.0 (-4.2 to 6.0)	.95
Pre-FEV ₁ (L) (cases = 135, controls = 46)	-0.21	0.09	.10
Post-FEV ₁ (L) (cases = 125, controls = 32)	-0.07	-0.09	.93
FEV ₁ reversibility (% change)	4.0 (-3.0 to 12.0)	0.0 (-7.0 to 6.2)	.01
Pre-FEV _{0.5} (L) (cases = 41, controls = 9)	-0.02	0.24	.04
Post-FEV _{0.5} (L) (cases = 37, controls = 9)	0.32	0.18	.33
FEV _{0.5} reversibility (% change)	5.0 (-3.0 to 13.5)	4.0 (-4.0 to 16.0)	.98
Pre-FEF ₅₀ (L/s) (cases = 115, controls = 22)	-0.03	0.15	.20
Post-FEF ₅₀ (L/s) (cases = 106, controls = 22)	-0.08	0.05	.43
FEF ₅₀ reversibility (% change)	15.0 (1.0-27.0)	9.0 (-11.5 to 34.0)	.70
FeNO (ppb), median (IQR) (cases = 133, controls = 35)	3 (0-8)	2 (0-5)	.16

PEF, peak expiratory flow; FVC, forced vital capacity; FEV₁, forced expiratory volume in the first second; FEV_{0.5}, forced expiratory volume in 0.5 s; FEF 50, forced expiratory flow in the mid of the FVC effort; FeNO, fractional exhaled nitric oxide.

bold values are statistical significant.

were more prevalent in the asthmatic group, a characteristic well described in this age group.²³ Increased television attendance time was noted in the asthmatics, despite no reported differences in physical activity, although a vicious circle of physical activity limitation and reduced fitness in asthmatics has previously been shown.²⁴ The asthmatic children in the PreDicta cohort were significantly more exposed to tobacco smoke at home, which has well been established as a major trigger for increased respiratory symptomatology.²⁵

Allergic sensitization was expectedly higher in the cases, closely resembling proportions in previous studies such as the COAST and PIAMA cohorts (58% and 35%-46% for the asthmatics and the controls in preschoolers, respectively)^{26,27} and from general population.²⁸ Early and persistent sensitization, more so to

inhalant allergens, has been significantly associated with asthma persistence,²⁹ although such relationship is not dichotomous and can be influenced by quantitative and qualitative measurements of allergic sensitization.³⁰

Allergic rhinitis was frequently diagnosed in the PreDicta cohort, especially in the asthmatics in agreement with previous studies.³¹ Furthermore, a high proportion of the asthmatics had a history of atopic dermatitis (51.5% vs 6.1% in the controls), supporting the general concept of the allergic march.

Viral respiratory infections are the most common trigger for asthma worsening in our cohort, consistent with several studies showing the role of—mostly RV—infection in asthma development and exacerbations.^{26,32} Asthma morbidity was significantly increased during fall, in accordance with the well-described “September

epidemic",³³ although morbidity appeared to be protracted throughout fall and winter.

An increased number and duration of antibiotic courses were prescribed in the asthmatics in the PreDicta cohort, as was previously shown more so in asthmatic preschoolers, potentially due to prolonged and recurrent respiratory symptoms.³⁴

With respect to lung function and airway inflammation, measurements were within normal limits, although the cases presented

lower values than the controls, in line with previous studies also reporting normal values by means of spirometry and FeNO measures in asthmatic children with well-controlled asthma.³⁵

Comparison between centers showed that prescription practices and asthma phenotyping according to trigger differed between centers, probably due to differences in viral and allergy season epidemiology.³⁶

To phenotype children, a data-driven approach with an unsupervised statistical learning technique identified four clusters, including one control and three asthmatics, with distinct respiratory allergy activity characteristics. Previously, hypothesis-free statistical techniques had also identified asthmatic groups with distinct activity and frequency of allergy-associated diseases, that is, when applied in the MeDALL cohorts.³⁷

Our study has several strengths, including its multinational character, as children were recruited from well-distributed European regions with differences in climate and asthma prevalence. Diagnosis and disease assessment were made by specialized doctors, while lung function, inflammation, hyperresponsiveness, and atopy were quantified via objective standardized measures. Moreover, our telemedicine system enabled us to avoid recall bias and to monitor the activity of the disease and the patients' compliance on a real-time basis. The cohort is representative of preschool asthma, with well-balanced demographic and atopy-related characteristics, and is well powered to address our primary hypothesis.

Nevertheless, a number of limitations have to be considered. The size of the cohort is moderate, and therefore, assessment of

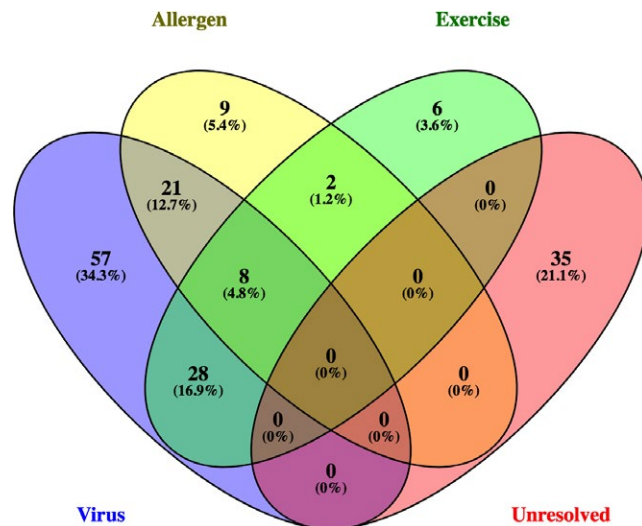


FIGURE 2 Venn diagram of asthma classifications according to trigger [Colour figure can be viewed at [wileyonlinelibrary.com](#)]

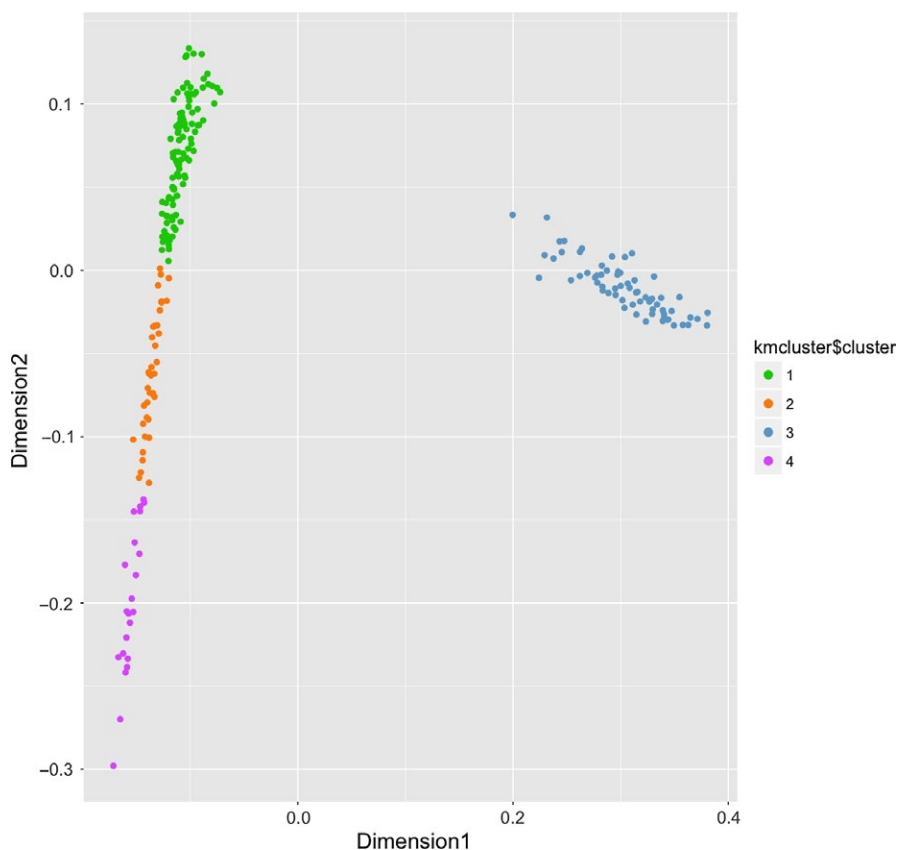


FIGURE 3 Multidimensional scaling plot that includes 3 clusters in the cases dataset and one for the controls [Colour figure can be viewed at [wileyonlinelibrary.com](#)]

TABLE 5 Characteristics of the clusters derived from the unsupervised analysis

Parameters	Cluster 3, n = 29 mild disease activity (%)	Cluster 1, n = 85 moderate disease activity (%)	Cluster 2, n = 46 high disease activity (%)	P value
Diagnosed allergic rhinitis	7.5	30.6	54.3	.03
Asthma control				
Controlled	87.5	41.1	35.3	.01
Partly controlled	12.5	42.9	47.1	
Uncontrolled	0.0	16.1	17.6	
Asthma severity				
Intermittent, n (%)	75.0	19.6	20.6	<.001
Mild persistent	25.0	67.9	52.9	
Moderate persistent	0.0	12.5	26.5	
Day asthma symptoms in the last 3 mo				
<1/wk	81.3	60.7	47.1	.06
>1/wk but <1/d	18.8	21.4	41.2	
daily	0.0	9.4	11.8	
Completely well between asthma episodes	93.8	64.3	73.5	.06
Number of asthma episodes in the last 12 mo	37.5	71.4	76.5	.01
Inhaled corticosteroids (yes), n (%)	68.7	64.3	82.4	.01
Pre-FEV _{0.5} (median, CI), L	1.0 (1.0-1.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	.05
Pre-FVC (median, CI), L	1.0 (1.0-1.0)	1.00 (1.0-1.0)	1.0 (1.0-1.0)	.06
Number of hospitalizations (median, CI)	0.0 (0.0-0.75)	0.0 (0.0-0.75)	0.0 (0.0-0.0)	.06
Mother with eczema	27.6	12.9	6.5	.03
Allergic sensitization (at least one positive SPT)	62.1	40.0	52.2	.09

bold values are statistical significant.

risk factors other than repeated infections can only be exploratory. Moreover, asthma diagnosis can be challenging at this age group, as no objective criteria can be applied. Several parameters, including asthma activity, severity, and medication use, were based on parental reports, which are subject to recall bias, although previous studies have validated that at least short-term parental reports can be accurate.³⁸ Unavoidably, a large proportion of children received asthma prophylactic treatment, a fact that may affect phenotyping and infection-related outcomes. Although a marginally high drop-out rate of 16.7% was noted for the cohort in total, this was within the accepted limits of the power analysis. Nevertheless, a more

prolonged follow-up period would potentially assist in discriminating the course of the disease more appropriately.

In conclusion, the PreDicta pediatric cohort was set to prospectively characterize asthma persistence in relation to repeated infections and atopy, also collecting multiple measurements on immune and inflammatory responses, viral infections, bacterial colonization, lung function, and airway inflammation, while disease activity was assessed by intensive follow-up. The results of this study are expected to clarify the role of respiratory viral infections in asthma persistence, in association with the underlying immune responses.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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Figure S1. Importance of parameters tested, separating distances between patients by a. mean decrease accuracy and b. mean decrease Gini

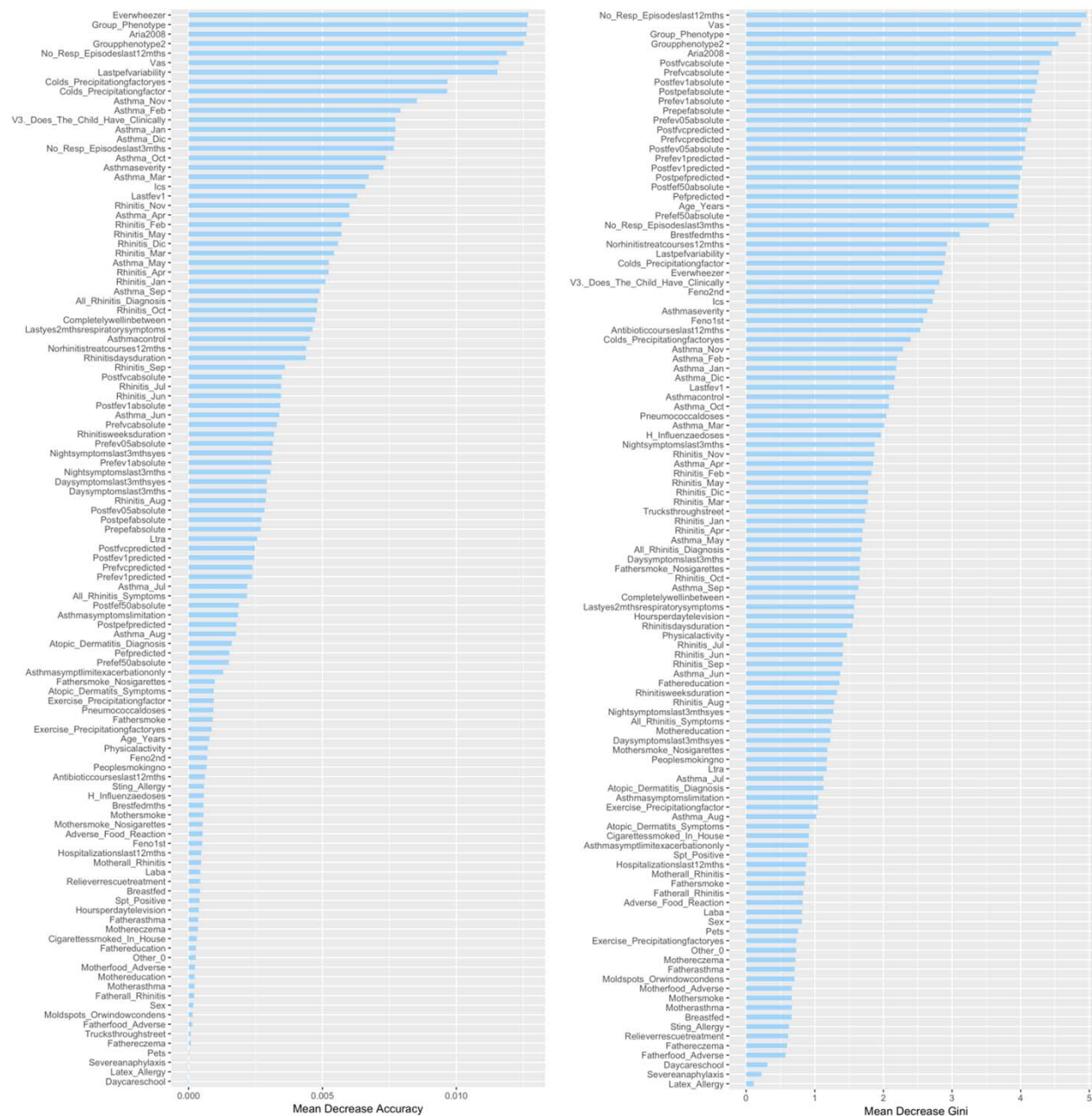


Figure S2. Silhouette width plots showing the optimum number of clusters for all tested clustering algorithms.

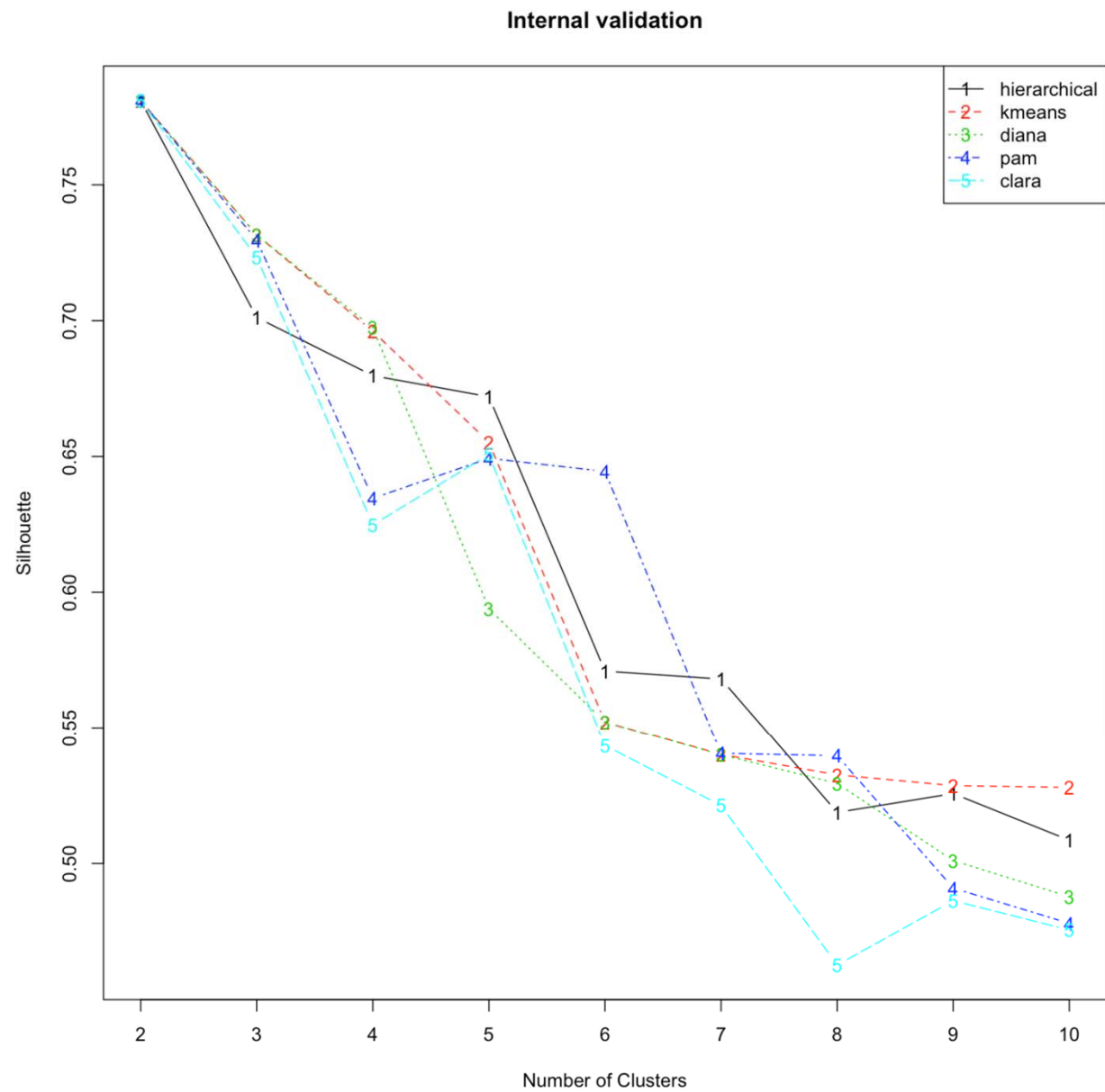


Figure S3. Cases and controls distinct separation in the multidimensional plot of the random forest dissimilarity

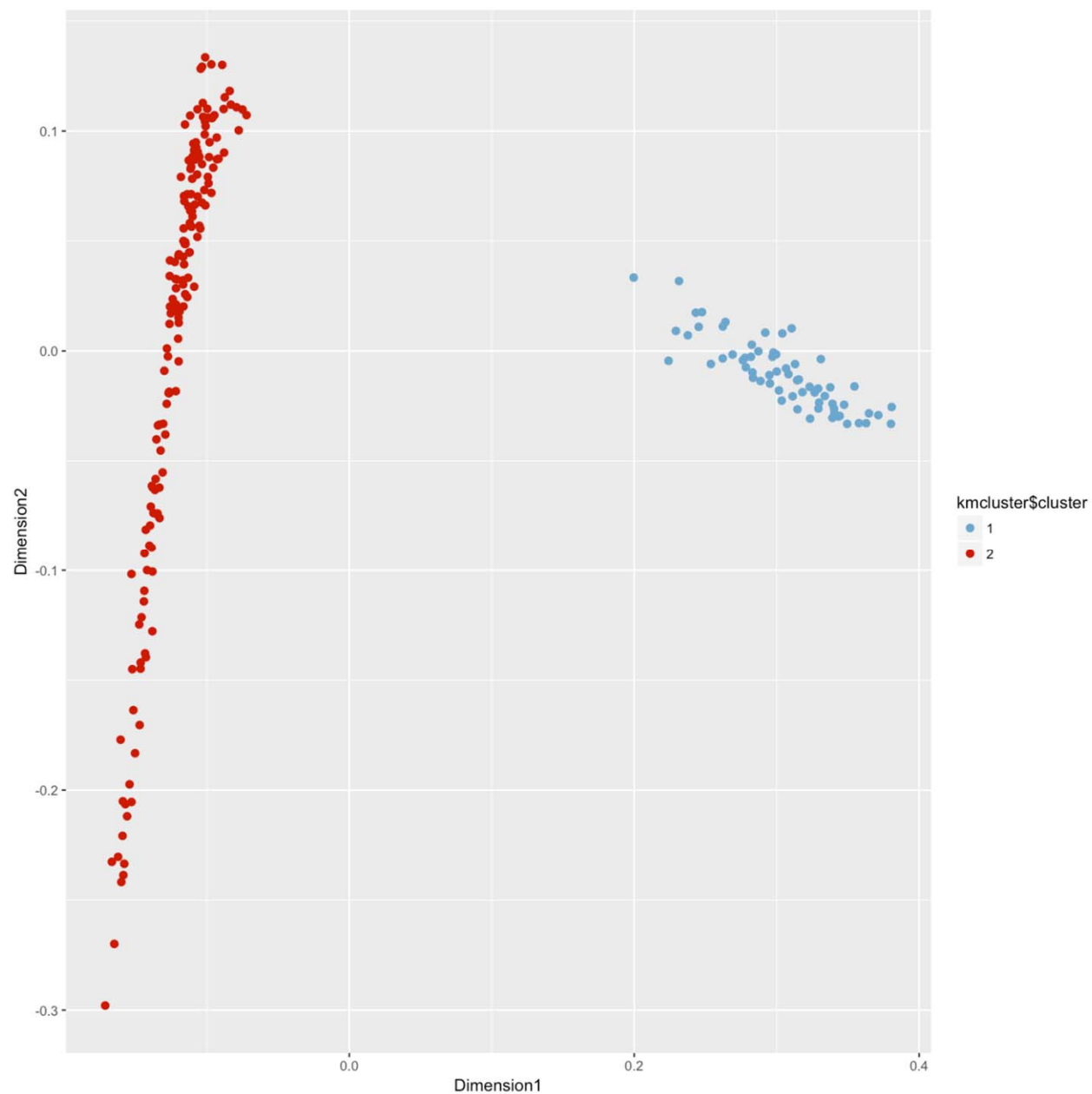


Figure S4. Importance of parameters tested, separating distances between cases by a. mean decrease accuracy and b. mean decrease Gini

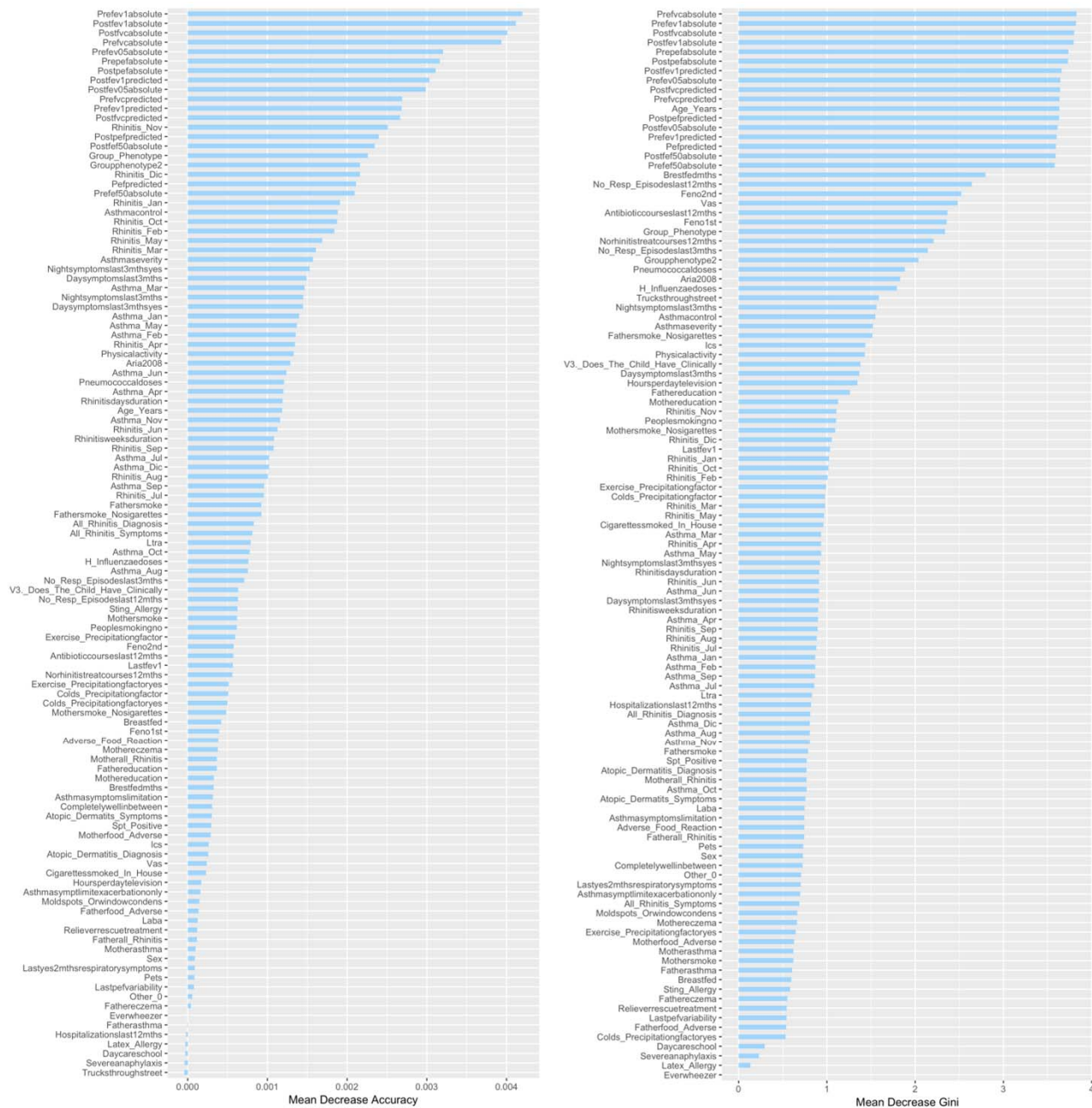


Figure S4. Silhouette width plots showing that the optimum clusters of cases is three

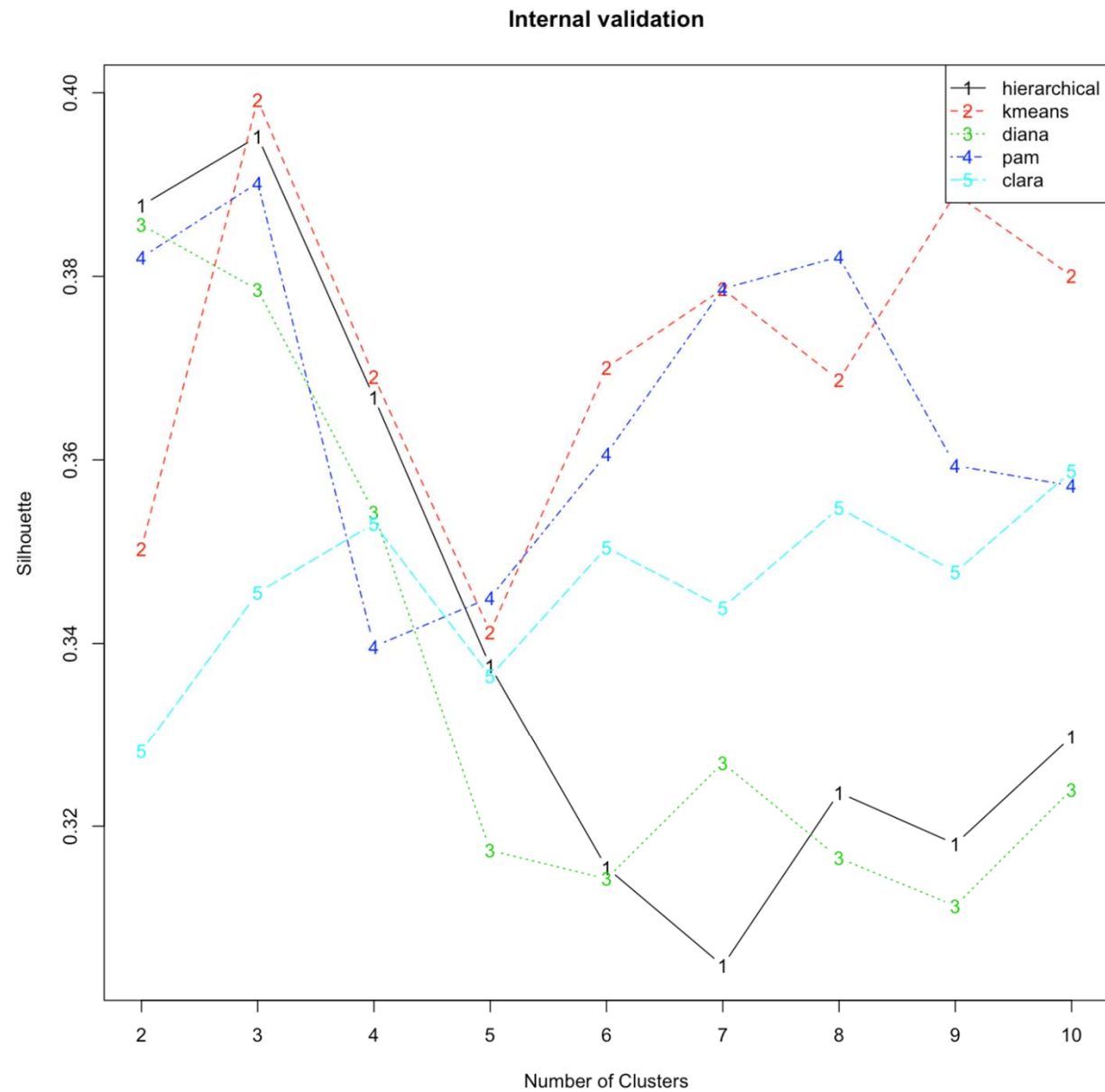


Table S1. Differences in baseline characteristics between centers (only statistically significant ones are shown)

	Athens n (%)	Erlangen n (%)	Ghent n (%)	Lodz n (%)	Turku n (%)	p value
<u>Asthma trigger</u>						
Virus	36 (97)	17 (71)	4 (18)	40 (97)	27 (37)	<0.001
Allergen	2 (5.4)	6 (25)	8 (36)	22 (54)	2 (4.5)	
Exercise	17 (46)	4 (16.5)	3 (13.5)	17 (41.5)	3 (7.0)	
Unresolved	1 (2.7)	4 (16.7)	9 (40.9)	1 (2.4)	23 (53.5)	
<u>Level of asthma severity</u>						
Intermittent	2 (5.4)	15 (62)	13 (59)	0 (0)	20 (46.5)	<0.001
Mild persistent	32 (86.5)	6 (25)	7 (32)	28 (68)	19 (44)	
Moderate persistent	3 (8)	3 (12.5)	2 (9)	13 (32)	4 (9)	
<u>Level of asthma control</u>						
Controlled	15 (40.5)	14 (58)	15 (68)	7 (17)	32 (74)	<0.001
Partly controlled	19 (51)	8 (33)	7 (32)	17 (41.5)	10 (23)	
Uncontrolled	3 (8)	2 (8)	0 (0)	17 (41.5)	1 (2)	
<u>Medication Use</u>						
Regular ICS	19 (51.5)	19 (79)	17 (77)	36 (88)	42 (98)	<0.001
Montelukast	23 (62)	2 (8)	4 (18)	31 (75.5)	4 (9)	<0.001

N, % = number and proportion of asthmatic children within centers

Table S2. Samples collected in the asthmatics and the controls at baseline in the PreDicta cohort

Peripheral blood (n=167 cases, 66 controls)	Whole blood (n=167 cases, 66 controls)	Nasopharyngeal samples (n=166 cases, 66 controls)
Serum	epigenetic and transcriptomic profiling	Viral detection
Antibody determination for rhinoviruses	Cytokine responses from peripheral mononuclear cell cultures to various stimuli	Bacterial detection
Determination of lipid mediators (resolvins, protectins, lipoxins)		
Atopy assessment (MeDALL- chip)		
Staphylococcus specific-IgE		
Vitamin D		

