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Correlation Of IL-5 And IL-17 With Specific Allergens in Pediatric Asthmatic Patients

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Abstract

Background: This study aimed to investigate the correlation between interleukin-5 (IL-5) and interleukin-17 (IL-17) with specific aeroallergens in pediatric asthmatic patients.

Patients and Methods: A cross-sectional study included (100) pediatric asthmatic patients, focusing on demographics, Biomarkers, and aeroallergens.

Results: The results showed that eosinophilic asthma patients had considerably higher levels of IL-5 (p=0.0015), especially those who were sensitized to allergens like sorrel, white ash, and sweet vernal mix. IL-17 levels were associated with sensitivity to firebush and CCD indicators and were higher in neutrophilic asthma (p=0.0072). Overlapping biomarker elevations in mixed granulocytic asthma suggested intricate inflammatory processes.

Conclusion: The significance of IL-5 and IL-17 as biomarkers for asthma phenotype in juvenile patients is highlighted by this study. IL-5 plays a crucial role in allergen-induced asthma, as seen by the strong association it has with particular allergens. Asthma treatment could be completely transformed by personalized therapy based on biomarker analysis.

العلاقة بين IL-5 وIL-17 ومسببات الحساسية المحددة لدى مرضى الربو الأطفال

خالد جبار سالم، الاء سعد العتابي، ضمياء مكي حمزة، حيدر عبد الامير نجم عبود

الخلاصة

المقدمة

هدفت هذه الدراسة إلى دراسة العلاقة بين الإنترلوكين-5 (IL-5) والإنترلوكين-17 (IL-17) مع مسببات الحساسية الجوية المحددة لدى مرضى الربو الأطفال.

المرضى وطرق العمل

شملت هذه الدراسة المقطعية (100) مريضًا بالربو من الأطفال، مع التركيز على التركيبة السكانية والعلامات الحيوية ومسببات الحساسية الجوية.

النتائج

أظهرت النتائج أن مرضى الربو الحمضي لديهم مستويات أعلى بكثير من IL-5 (p=0.0015)، وخاصة أولئك الذين كانوا حساسين لمسببات الحساسية مثل الحميض والرماد الأبيض ومزيج الربيع الحلو. ارتبطت مستويات IL-17 بالحساسية لمؤشرات شجيرة النار وCCD وكانت أعلى في الربو العدلي (p=0.0072). تشير ارتفاعات المؤشرات الحيوية المتداخلة في الربو الحبيبي المختلط إلى عمليات التهابية معقدة.

الاستنتاج

وُجِد في هذه الدراسة أهمية IL-5 وIL-17 كعلامات حيوية لنمط الربو لدى المرضى الأحداث. يلعب IL-5 دورًا حاسمًا في الربو الناجم عن المواد المسببة للحساسية، كما يتضح من الارتباط القوي الذي له بمسببات الحساسية المحددة. يمكن تحويل علاج الربو تمامًا من خلال العلاج الشخصي القائم على تحليل العلامات الحيوية.

1. Introduction

Asthma, a chronic respiratory disorder that affecting over 300 million individuals worldwide, is still particularly prevalent among children with significant morbidity and economic burden (Levy *et al.*, 2023). This disorder condition is characterized by hyper responsiveness of airway, obstruction, and persistent inflammation driven by diverse immunological pathways. In pediatric; asthma often manifested in distinct phenotypes, including eosinophilic and neutrophilic asthma each of them associated with the inflammatory profiles (Wenzel, 2012). Interleukin-5 (IL-5) a cytokine pivotal to eosinophilic inflammation that promoting eosinophil differentiation with activation and survival (Takatsu, 2011). In the context, interleukin-17 (IL-17) Produced by Th17 cells, IL-17 has been implicated in neutrophilic asthma and associated with the severe and steroid-resistant asthma in patients (Rahmawati *et al.*, 2021). Association of biomarkers with environmental allergens, such as pollen and animal dander were recognized as primary triggers for asthma exacerbations especially in atopic individuals. Interleukin-5 is closely associated with to allergen driven eosinophilic inflammation, while interleukin-17 had role extends to non-atopic triggers such as pollution and infections (Hammad and Lambrecht, 2021). This study aims to investigate the association between IL-5, IL-17, and specific allergens to enhance asthma diagnosis and allocate appropriate treatments.

2. Material and Methods

2.1. Study Design

A cross-sectional study included 100 pediatric asthmatic patients aged from 6 to 15 years. Participants were divided into four group asthma phenotypes: (eosinophilic, neutrophilic, mixed granulocytic, and allergic asthma).

2.2. Inclusion Criteria

1. Diagnosed asthmatic children aged (6-15) years.
2. Children is receiving inhaled corticosteroids.

2.3. Exclusion Criteria

1. Patients on systemic corticosteroids or biologic therapy.
2. All patients' upper and lower respiratory infection diseases.
3. Patients with autoimmune disease and these with cystic fibrosis also should be excluded.

2.4. Data Collection and Analysis

1. Demographic and Clinical data will be collected using a specific detailed questionnaire
2. Approximately 5 ml of venous blood were drawn from each subject which were obtained by disinfecting antecubital fossa with 70% ethanol and then make vein puncture by disposal syringes after applying a tourniquet. One ml of blood was dispensed into (1) EDTA tube for the haematological tests. Four ml of blood was dispensed into gel tube and allowed to clot then serum was separated by centrifugation at

3000 round per minutes (RPM) for 5 minutes. Then the serum was transferred to new eppendorf tube (2 ml) and stored in deep freeze (-20°C) to be used for immunological assays.

3. Immunological measurement of IL-5, IL-17, and specific IgE (S.IgE) by ELISA Kit
4. The study was used Enzyme-linked immunosorbent assay (ELISA): for biomarkers IL5, IL17 ,total IgE (T.IgE) and specific immunoglobulin E (S.IgE) inhalation in peripheral blood for all patients
5. Correlation between biomarkers and allergen sensitivity was analyzed.

2.5. Ethical Considerations

Ethical approval was obtained from the Karbala College of Medicine and patient consent was secured.

2.6. Statistical Analysis

Statistical analysis of data in the present study was performed by SPSS. version 25.0 on the basis of one way analysis of variance (ANOVA) using significant levels ($P<0.05$).

3. Results

3.1. Demographic Data for Asthma Patients

The demographic data of asthmatic patients were included in current study were illustrated in Table (3-1). These data were involved: age, sex, residency, passive smoking, family history, Food allergy, Allergic Rhinitis, and Pets at home. patients were divided according to their ages into three groups: 5-8 y, 9-12 y, and 13-16 y, the results of statistical analysis using Chi-square test revealed a highly significant ($p=0.0001$) differences between groups, where the highest percent (58.0%) of patients within the age group 9-12 y. The majority (72.5%) of asthma patients were male, while only (27.5%) were female, the results of statistical analysis showed a highly significant ($p=0.0001$) difference. In the context of residency, a significantly ($p=0.0009$) highest percent (66.4%) of patients were resided in urban, while only (33.6%) resided in rural. As for passive smoking, patients were divided into two groups: non-passive smoking and passive smoking, but statistical analysis did not show any significant differences between the two groups; where $p=0.3173$. Regarding family history; significantly ($p=0.0001$), most patients (70.2%) had a family history of asthma, while only 29.8% had no family history of the disease. As for food allergy, patients were divided into two groups according to present or absent food allergy. The results of the statistical analysis showed that the majority (82.4%) of patients in the current study did not have food allergy, while the smaller percentage (17.6%) had a food allergy; $p=0.0001$. with respect to Allergic Rhinitis and presence a pet at home, the results of statistical analysis found non-significant ($p>0.05$) differences between groups of asthma patients divided according to these mentioned characteristics as, as shown in Table1.

Table1: Demographic Data for Asthma Patients

Age group (year)					
	5-8 y	**9-12 y**	**13-16 y**	**Total**	***P value***
Count (%)	29(29%)	59(59%)	12(12%)		0.0001*
Mean ± SD	9.71 ± 2.328				
Gender					
	Male	**Female**		**Total**	***P value***
Count (%)	75 (75%)	25(25%)		100	0.0001*
Residency					
	Urban	**Rural**		**Total**	***P value***
Count (%)	65 (65%)	35(35%)		100	0.0027*
Passive Smoking					
	Non-2nd smoking	**2nd smoking**		**Total**	***P value***
Count (%)	52(52%)	48 (48%)		100	0.6892
Family Hx					
	Non- Family hX	**Family hX**		**Total**	***P value***
Count (%)	30 (30%)	70 (70%)		100	0.0001*
Food allergy					
	Non- food allergy	**Food allergy**		**Total**	***P value***
Count (%)	82(82%)	18 (18%)		100	0.0001*
Allergic Rhinitis					
	Non-allergic Rhinitis	**Allergic Rhinitis**		**Total**	***P value***
Count (%)	43(43%)	57 (57%)		100	0.1615
Pets at home					
	No	**Yes**		**Total**	***P value***
Count (%)	48(48%)	52(52%)		100	0.6892
*Mean significant difference P< 0.05 level by chi-square test					

3.2. Biomarkers Concentrations in Asthma Patients

3.2.1. Biomarkers Concentrations in Asthma Patients According to Eosinophilic Group

Table2 displays the concentrations of studied markers (IL-17, IL-5) according to Eosinophilic group (Eosinophil scores) in asthmatic patients, which divided into three groups: high, low and normal. Statistical analysis using one way-ANOVA test showed non-significant ($p>0.05$) differences in the distributions of IL-17, while highly significant ($P=0.0015$ and $P=0.0054$ respectively) for IL-5.

Table2: Biomarkers Concentrations in Asthma Patients According to Eosinophil Scores

Biomarkers/units	Eosinophil level	No.	Biomarker concentration in patients		P value
			Mean	Std. Deviation	
IL-17 (unit)	High	65	221.54969	112.862866	0.212
	Low	14	184.11750	108.190582	
	Normal	21	255.21229	133.607551	
	Total	100	223.37833	117.529254	
IL-5 (unit)	High	65	262.74848	194.840479	**0.0015***
	Low	14	142.91371	45.396255	
	Normal	21	196.93794	141.376216	
	Total	100	203.19476	148.838171	
*Mean significant difference $p \leq 0.05$ by One way – ANOVA					

3.2.2.Biomarkers Concentrations in Asthma Patients According to Neutrophil Scores

Table3 shows the concentrations of studied markers (IL-17, IL-5) according to Neutrophil scores in asthma patients, which divided into three groups: high, low and normal. Statistical analysis using one way-ANOVA test showed highly-significant (P=0.0072 respectively) for IL-17 while for these markers IL-5 there was no significant

Table3: Biomarker Concentration in Patients According to Neutrophil Levels

Biomarkers/units	Neutrophil level	No.	Biomarker concentration in patients		P value
			Mean	Std. Deviation	
IL-17 (unit)	High	32	200.51786	288.88537	**0.0072***
	Low	34	153.85226	218.18139	
	Normal	34	195.29070	285.54717	
	Total	100	200.05798	246.69868	
IL-5 (unit)	High	32	167.40991	412.99727	0.416
	Low	34	124.63665	536.83347	
	Normal	34	89.64867	302.64733	
	Total	100	187.51196	354.87756	
*Mean significant difference $p \leq 0.05$ by One way – ANOVA					

3.2.3.Biomarkers Concentrations in Asthma Patients According to Mix Granulocytic Score

Table4 shows the concentrations of studied markers (IL-17, IL-5) according to mix granulocytic score in asthmatic patients, which divided into two groups: Mix and non-mix. Statistical analysis using one way-ANOVA test showed highly-significant (P=0.0074, P=0.0021) for IL-17, IL-5.

Table4: Biomarker Concentration in Patients According to Mix Granulocytic Score

Biomarkers/units	mix granulocytic score	No.	Biomarker concentration in patients		P value
			Mean	Std. Deviation	
IL-17	Mix	22	274.02077	134.435195	0.0074*
	Non	78	209.09456	109.047451	
	Total	100	223.37833	117.529254	
IL-5	Mix	22	412.86282	600.165762	0.0021*
	Non	78	231.23710	350.852946	
	Total	100	271.19476	421.742178	
*Mean significant difference p ≤ 0.05 by One way – ANOVA					

3.4. Frequencies of All Aeroallergens (Aag) in Asthma Patients

The frequencies of all (Aag) in asthma patients were illustrated in Table5. The results of the statistical analysis using chi-square test, indicated that the percentages of patients without antigens were significantly (p=0.0001) higher than those with (Aag), and this applies to all (Aag) included in the current study. The highest frequency (Aag) among asthma patients in the current study were Cat (18%), cultivated oat (17%) Meadow foxtail (19%), Goosefoot (15%), Russian thistle (22%), Rough pigweed (16%), Cockroach Germany (9%) and Altrenaria Altrenaria(12%)

Table5: Frequency of all Aeroallergens in Asthma Patients

Aeroallergens		Count	%	Total	P value
Cat	Positive	18	18%	100	0.0001
	Negative	82	8 %		
Cultivated oat	Positive	17	17%	100	0.0001
	Negative	83	83%		
Meadow foxtail	Positive	19	19%	100	0.0001
	Negative	81	81%		
Goosefoot	Positive	15	15%	100	0.0001
	Negative	85	85%		
Russian thistle	Positive	22	22%	100	0.0001
	Negative	78	78%		
Rough pigweed	Positive	16	16%	100	0.0001
	Negative	84	84%		
Cockroach Germany	Positive	9	9%	100	0.0001
	Negative	91	91%		
Alternaria	Positive	12	12%	100	0.0001
	Negative	88	88%		
Significant difference P< 0.05 level by chi-square test					

3.3. Biomarkers Concentration in Patients According to Aeroallergens

3.3.1. Biomarkers Concentration in Patients According to Aeroallergens (Sweet Vernal Mix) .

Table6 shows the effects of Aeroallergen (Sweet vernal mix) on the biomarker's concentration of asthmatic patients. The results of the statistical analysis showed that highly significant in IL-5 (P=0.002), were is it increased in patients that have inhaled (positive) Aeroallergen (Sweet vernal mix). While other remaining biomarkers did not show any significant IL-17 (P=0.421).

Table6: Biomarkers Concentration in Patients According to Aeroallergens (Sweet Vernal Mix)

Biomarkers/units	Aeroallergen (Sweet vernal mix)	N	Biomarker concentration in patients		P value
			Mean	Std. Deviation	
IL-17	Positive	13	219.70298	115.652329	0.421
	Negative	78	247.97492	131.709794	
	Total	100	223.37833	117.529254	
IL-5	Positive	13	597.63077	861.481380	**0.002***
	Negative	78	222.41697	287.545651	
	Total	100	271.19476	421.742178	
* Significant difference p ≤ 0.05 by One way – ANOVA					

3.3.2. Biomarkers Concentration in Patients According to Aeroallergens (Firebush).

Table7 shows the effects of Aeroallergen (Sweet vernal mix) on the biomarker's concentration of asthmatic patients. The results of the statistical analysis showed that significantly in biomarkers IL-17 (P=0.003 respectively), were is it increased in patients that have inhaled (positive) Aeroallergen (Firebush). While the other remaining biomarkers did not show any significant IL-5 (P=0.210)

Table7: Biomarkers Concentration in Patients According to Aeroallergens (Firebush)

Biomarkers/units	Aeroallergen/(Firebush)	N	Biomarker concentration In patients		P value
			Mean	Std. Deviation	
IL-17	Positive	10	231.52541	118.741221	**0.003***
	Negative	90	150.05460	76.672808	
	Total	100	221.38722	113.128273	
IL-5	Positive	10	111.88650	49.079889	0.210
	Negative	90	288.89568	440.952878	
	Total	100	261.17474	430.651267	
*** Significant difference under p ≤ 0.05 by One way – ANOVA**					

3.3.3. Biomarkers Concentration in Patients According to Aeroallergens (Sorrel)

Table8 shows the effects of Aeroallergen (Sorrel) on the biomarker's concentration of asthmatic patients. The results of the statistical analysis showed that significantly only in IL-5 (P=0.004), were is it increased in patients that have inhaled (positive) Aeroallergen (Sorrel). While the other remaining biomarkers did not show any significant SAA1 and IL-17 (P=0.317 and P=0.244)

Table8: Biomarkers Concentration in Patients According to Aeroallergens (Sorrel)

Biomarkers/units	Aeroallergen (Sorrel)	N	Biomarker concentration In patients		P value
			Mean	Std. Deviation	
IL-17	Positive	4	220.57337	116.632690	0.244
	Negative	96	290.69725	137.008823	
	Total	100	203.24836	107.332244	
IL-5	Positive	4	684.58900	1026.119471	**0.004***
	Negative	96	253.97000	380.276282	
	Total	100	273.17446	441.766168	
* Significant difference $p \leq 0.05$ by One way – ANOVA					

3.3.4. Biomarkers Concentration in Patients According to Aeroallergens (White Ash)

Table9 shows the effects of Aeroallergen (White ash) on the biomarker's concentration of asthmatic patients. The results of the statistical analysis showed that highly significant in IL-5 (P=0.006), were is it increased in patients that have inhaled (positive) Aeroallergen (White ash). While the other remaining biomarkers did not show any significant IL-17 (P=0.306)

Table9: Biomarkers Concentration in Patients According to Aeroallergens (White Ash)

Biomarkers/units	Aeroallergen (White ash)	No.	Biomarker concentration in patients		P value
			Mean	Std. Deviation	
IL-17	Positive	7	220.05989	115.792100	0.306
	Negative	93	267.46614	141.038177	
	Total	100	221.656315	113.459334	
IL-5	Positive	7	688.49429	936.238586	**0.006***
	Negative	93	239.78512	346.391350	
	Total	100	241.29467	454.743848	
* Significant difference $p \leq 0.05$ by One way – ANOVA					

3.3.5. Biomarkers Concentration in Patients According to Aeroallergens (Tree Mix 4)

Table10 shows the effects of Aeroallergen (Tree Mix 4) on the biomarker's concentration of asthmatic patients. The results of the statistical analysis showed that significantly in IL-5 (P=0.005), were is it increased in patients that have inhaled (positive) Aeroallergen (Tree Mix 4). While the other remaining biomarkers did not show any significant IL-17 (P=0.640).

Table10: Biomarkers Concentration in Patients According to Aeroallergens (Tree Mix 4)

Biomarkers/units	Aeroallergen (Tree Mix 4)	N	Biomarker concentration In patients		P value
			Mean	Std. Deviation	
IL-17	Positive	4	222.24922	117.558341	0.640
	Negative	96	250.47700	131.076512	
	Total	100	213.67599	117.765844	
IL-5	Positive	4	673.42075	1168.957312	**0.005***
	Negative	96	254.43534	367.570818	
	Total	100	272.19825	411.747654	
* Significant difference $p \leq 0.05$ by One way – ANOVA					

3.3.6. Biomarkers Concentration in Patients According to Aeroallergens (CCD Markers)

Table11 shows the effects of Aeroallergen (CCD markers) on the biomarker's concentration of asthmatic patients. The results of the statistical analysis showed that significantly in IL17 (P=0.005), were is it increased in patients that have inhaled (positive) Aeroallergen (CCD markers). While the IL-5 did not show any significant (P=0.169).

Table11: Biomarkers Concentration in Patients According to Aeroallergens (CCD Markers)

Biomarkers/units	Aeroallergen (CCD markers)	N	Biomarker concentration in patients		P value
			Mean	Std. Deviation	
IL-17	Positive	**4**	335.19400	90.970187	**0.005***
	Negative	**96**	218.71934	116.554841	
	Total	**100**	226.74823	117.67214	
IL-5	Positive	**4**	259.32618	408.248584	0.169
	Negative	**96**	556.04075	692.183039	
	Total	**100**	270.19476	421.742178	
* Significant difference $p \leq 0.05$ by One way – ANOVA					

ROC Analysis Receiver operating characteristic (ROC) analysis demonstrated that IL-5 and periostin have high predictive potential for identifying eosinophilic asthma phenotypes in allergen-positive patients, Table12.

Table12: ROC Analysis of Biomarkers in Patients According to S. Ige

Biomarkers/units	AUC	Sensitivity	Specificity	Cut-off	P-value
IL-17	0.536	0.516	0.694	190.837	0.549
IL-5	0.569	0.406	0.75	168.474	0.251
* Significant difference $p \leq 0.05$ by One way – ANOVA					

4. Discussion

Demographic Characteristics and Asthma Prevalence

The current study's demographic analysis highlighted key factors influencing on asthma prevalence and severity in children. The significant proportion of asthmatic patients fall within the (9-12) years age group (58% with p=0.0001) suggesting potential vulnerability in this age group may be due to hormonal changes. The predominant of male patients (72.5%, p=0.0001) aligns with previous study that indicating that boys have tighter airways during early childhood, making them at risk to asthma exacerbation (Ricciardolo *et al.*, 2023). Regarding urban residency was significantly associated with asthma prevalence (65%, p=0.0009). This belongs to urban area had greater air pollutants and allergens. However, passive smoking has been a risk factor for respiratory and allergic conditions in children (Kim, Vazquez and Cubbin, 2023). A strong familial predisposition to asthma (70.2%, p=0.0001) caused by genetic component. This aligns with the findings of Cookson et al., (2011) who recognized genetic polymorphisms associated with IgE levels and airway inflammation. As well as, food allergies were less common among those patients (17.6%, p=0.0001) so that suggesting that aeroallergens rather than ingested allergens play a more important role in triggering asthma.

Biomarkers and Inflammatory Profiles

Elevated IL-5 levels in patients with high eosinophil scores (p=0.0015) explained its role in driving eosinophilic inflammation. IL-5 contributes to eosinophil differentiation and activation, leading to airway hyperresponsiveness and mucus overproduction. These findings confirmed the utility of IL-5-targeted therapies such as mepolizumab in managing eosinophilic asthma (Hammad and Lambrecht, 2021).

Neutrophilic Asthma: In line with its function in neutrophil recruitment and activation, IL-17 levels were considerably greater in individuals with high neutrophil scores (p=0.0072). For severe asthma phenotypes that frequently do not respond to corticosteroid treatment, this discovery is especially pertinent. For these individuals, targeted treatments like anti-IL-17 monoclonal antibodies could provide novel therapeutic options (Yang *et al.*, 2018). Mixed granulocytic asthma had a significantly higher IL-5 concentration (412.86 ± 600.17 units) than non-mixed phenotypes (231.23 ± 350.85 units, p = 0.0021). Although IL-5 is a marker of eosinophilic inflammation, its increased level in individuals with mixed phenotypes could be a reflection of the overlapping inflammatory processes that these patients have. Chu *et al.*, (2015) discovered that a significant decrease in lung activating and chemotactic cytokines, namely IL-17A and IL-5, was linked to the prevention of neutrophilic and eosinophilic inflammation.

Aeroallergen Sensitivity and Biomarker Expression

The study highlighted the significance of environmental triggers in asthma exacerbations by identifying a number of aeroallergens with strong correlations to biomarker levels. IL-5 with Aeroallergens: Patients who were exposed to aeroallergens such white ash (p=0.006), sorrel (p=0.004), and sweet vernal mix (p=0.002) had higher levels of IL-5. According to these results, these allergens are important targets for allergen-specific immunotherapy as they appear to be the main cause of eosinophilic inflammation. IL-17 and Aeroallergens: Patients who were sensitive to firebush (p=0.003) and CCD indicators (p=0.005) had significantly higher levels of IL-17, suggesting that these allergens play a part in neutrophilic or mixed asthma phenotypes. Increased IL-17 in response to these allergens indicates the activation of non-Th2 pathways, especially in instances of severe asthma.

Limitations and Future Directions

The study highlighted the significance of environmental triggers in asthma exacerbations by identifying a number of aeroallergens with strong correlations to biomarker levels. IL-5 with Aeroallergens: Patients who were exposed to aeroallergens such white ash (p=0.006), sorrel (p=0.004), and sweet vernal mix (p=0.002) had higher levels of IL-5. According to these results, these allergens are important targets for allergen-specific immunotherapy as they appear to be the main cause of eosinophilic inflammation. IL-17 and Aeroallergens: Patients who were sensitive to firebush (p=0.003) and CCD indicators (p=0.005) had significantly higher levels of IL-17, suggesting that these allergens play a part in neutrophilic or mixed asthma phenotypes. Increased IL-17 in response to these allergens indicates the activation of non-Th2 pathways, especially in instances of severe asthma. A relatively small sample size may have an impact on the findings' generalizability; the study was cross-sectional, which limited the capacity to establish causal links. Environmental factors that may affect asthma phenotypes, like as pollution and food, were not thoroughly examined.

5. Conclusion

The significance of IL-5 and IL-17 as biomarkers for asthma phenotype in juvenile patients is highlighted by this study. IL-5 plays a crucial role in allergen-induced asthma, as seen by the strong association it has with particular allergens. Asthma treatment could be completely transformed by personalized therapy based on biomarker analysis.

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