








ORIGINAL ARTICLE

Asthma and Lower Airway Disease

Circulating mast cell progenitors increase during natural birch pollen exposure in allergic asthma patients

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Abstract

Background: Mast cells (MCs) develop from a rare population of peripheral blood circulating MC progenitors (MCps). Here, we investigated whether the frequency of circulating MCps is altered in asthma patients sensitized to birch pollen during pollen season, compared to out of season.

Methods: Asthma patients were examined during birch pollen season in late April to early June (May), and out of season in November–January. Spirometry measurements, asthma and allergy-related symptoms, asthma control questionnaire (ACQ), and asthma control test (ACT) scores were assessed at both time points. The MCp frequency was determined by flow cytometry in ficoll-separated blood samples from patients with positive birch pollen-specific IgE, and analyzed in relation to basic and disease parameters.

Results: The frequency of MCps per liter of blood was higher in May than in November ($p = .004$), particularly in women ($p = .009$). Patients that reported moderate to severe asthma symptoms ($< .0001$), nose or eye symptoms ($p = .02$; $p = .01$), or reduced asthma control (higher ACQ, $p = .01$) had higher MCp frequency in May than those that did not report this. These associations remained significant after adjusting for sex and BMI. The change in asthma control to a lower ACT score in May correlated with an increase in MCp frequency in May ($p = .006$, $\rho = 0.46$).

Conclusions: The data suggest that the frequency of MCps increases in symptomatic patients with allergic asthma. Our results unravel a link between asthma symptoms and circulating MCps, and bring new insight into the impact of natural allergen exposure on the expansion of MCs.

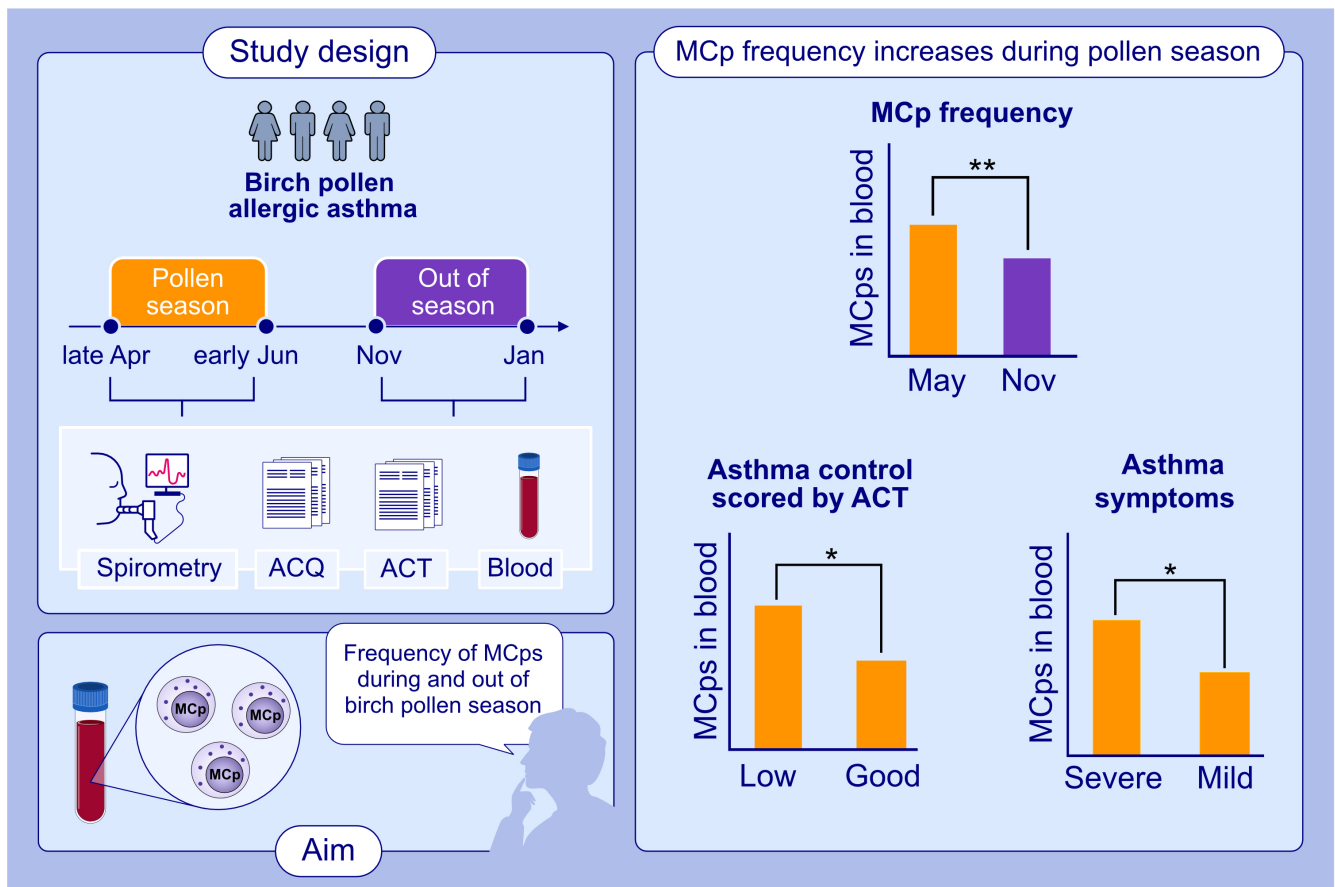
KEYWORDS

asthma control, asthma symptoms, *Betula*, FcεRI, mast cell

Abbreviations: ACQ, asthma control questionnaire; ACT, asthma control test; BMI, body mass index; FeNO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in 1 s; MC, mast cell; MCp, mast cell progenitor; PBMCs, peripheral blood mononuclear cells; PEF, peak expiratory flow.

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GRAPHICAL ABSTRACT

The frequency of blood circulating mast cells progenitors (MCps) was investigated in birch-pollen sensitized asthma patients during birch pollen season and out of the pollen season. The MCp frequency was higher in pollen season than out of season. In pollen season, the patients with more asthma symptoms, and reduced asthma control had more circulating MCps.

Abbreviations: ACQ, asthma control questionnaire; ACT, asthma control test; MCp, mast cell progenitor

1 | INTRODUCTION

Asthma is a chronic respiratory disease characterized by airway obstruction and inflammation accompanied by symptoms such as cough, wheezing, and shortness of breath.¹ Monitoring asthma progression and response to treatment is crucial for achieving good disease control. Spirometry is often used to assess lung function by measuring airflow changes during forced expiration. Furthermore, self-reported questionnaires such as the asthma control test (ACT) or asthma control questionnaire (ACQ) provide vital information about asthma control.²

Each year seasonal pollen exposure triggers symptoms that affect the nose, or eyes of allergic patients disrupting their daily activities and producing detrimental effects on quality of life.^{3,4} Around 20% of patients with allergic rhinitis also experience asthma symptoms upon allergen exposure.⁵ In Europe, pollen-related allergy is common, and in Scandinavia, birch pollen (*Betula*) is one of the main allergens causing symptoms.^{6,7} In sensitized patients, allergen exposure causes mast cell (MC) activation via IgE-allergen crosslinking of the high-affinity receptor for IgE (FcεRI), and a consecutive release

of proteases, cytokines, histamine, and leukotrienes.⁸ These mediators are involved in airway hyperresponsiveness, mucus secretion, vasodilation, and increased vascular permeability, which leads to symptom progression.

MC accumulation in the airways, smooth muscles, and the airway epithelium, is a pathological sign of asthma.^{9–12} Furthermore, during pollen exposure, MC numbers increase in the nasal and bronchial epithelium, suggesting an increased MC expansion or migration to the sites where the allergen is present.^{13,14} MCs mature from MC progenitors (MCps), which have been shown to be recruited from the bone marrow via the blood to the lung in mouse models of allergic airway inflammation.^{15–18} In 2016, we were the first to report a rare population of human FcεRI-expressing MCps in blood circulation.¹⁹ We also demonstrated that a high MCp frequency in peripheral blood was associated with reduced lung function in a cohort of well-controlled patients with allergic asthma.^{19,20} However, whether MCp frequency is related to asthma symptoms and severity is still unknown.

In the current study, the aim was to investigate whether the MCp frequency is related to allergen exposure in patients with allergic asthma. We determined the frequency of circulating MCps in birch

pollen-sensitized asthma patients sampled during pollen season, when they commonly experienced more asthma symptoms, and out of season when the allergen is no longer present.

2 | METHODS

2.1 | Study population and hospital visits

The study was approved by the Uppsala Regional Ethics Review Board (2017/535). The study participants included adult individuals who were diagnosed with asthma by their primary care physician according to the GINA guidelines. Specifically, these individuals reported asthma symptoms during the birch pollen season. All patients gave their written informed consent. Seven patients were later excluded from the analyses in the present study since they displayed birch pollen-specific IgE below $0.35 \text{ kU}_A/\text{L}$. Exclusion criteria included active smoking, and patients with severe asthma. Patients that had cold symptoms within the past 4 weeks before the visit were excluded from the first visit (in that season), or the follow-up visit was delayed (out of season).

One paired set of peripheral blood samples per patient was collected during birch pollen season, the majority in May (three in late April and one in early June), and out of pollen season during November–January in the years 2018, 2019, 2021, or 2022. At each visit, a total of 20 mL of peripheral blood was collected in tubes containing ethylenediaminetetraacetic acid (EDTA) (BD Vacutainer, BD Bioscience) for enrichment of peripheral blood mononuclear cells (PBMCs). Additional blood samples were taken for preparation of serum for determination of birch pollen-specific IgE antibody levels.

2.2 | Assessment of pulmonary function and asthma control

The pulmonary function was assessed by spirometry (Masterscreen PFT, Carefusion) at baseline following the ATS/ERS guidelines²¹ to determine forced expiratory volume in 1 s (FEV_{1s}), $\text{FEV}_{1s}/\text{FVC}$ ratio, and peak expiratory flow (PEF). Bronchodilator tests were performed using 400 micrograms of salbutamol in a spacer. The fractional exhaled nitric oxide (FeNO) was measured using an electrochemical sensor (NIOX Vero) according to ATS/ERS guidelines.²²

Asthma, nose, or eye symptoms were recorded at each visit (0=none, 1=mild, 2=moderate, and 3=severe) and self-reported asthma limitations were evaluated using the asthma control test (ACT, 5–25 score) and the asthma control questionnaire (ACQ, 0–6 score). The magnitude of general symptoms (fatigue, difficulty concentrating, and reduced physical activity) were scored as 0=none, 1=mild, 2=moderate, and 3=severe. One patient did not answer the questions about asthma, nose, or eye symptoms during May and was therefore excluded from these analyses. Another patient did not

answer the questions regarding general symptoms and was therefore excluded from this analysis.

2.3 | Preparation of PBMCs

Blood collected in EDTA-coated tubes was diluted 1:2 with 2% heat-inactivated fetal calf serum (FCS, Sigma-Aldrich) in 0.1 M PBS pH 7.4 (FACS buffer). The mixture was transferred to a 50 mL SepMate™ tube (Stemcell Technologies) containing 15 mL of Ficoll-Paque Premium ($\rho = 1.076 \text{ g/mL}$) (GE Healthcare). The Ficoll–blood mix was centrifuged at 1200g for 10 min at 19°C. The resulting supernatant was washed twice with FACS buffer at 200g for 10 min at 4°C. The cells were counted in a hemocytometer chamber using trypan blue dye before they were stored in 10% dimethyl sulfoxide (DMSO, Sigma-Aldrich) in FCS at -80°C until analysis.

2.4 | Flow cytometry

The vital frozen PBMCs were thawed by adding pre-warmed (37°C) FACS buffer into the vial until the cells were thawed completely. The cells were spun at 400g for 5 min at 4°C before staining. The following conjugated anti-human antibodies were used and described as target (clone) -fluorochrome conjugate: CD4 (RPA-T4)–BV605, CD8 (RPA-T8)–BV605, CD19 (HIB19)–BV605, CD14 (M5E2)–Alexa700, CD13 (WM15)–PerCP–Cy5.5, CD117 (104D2)–BV421, CD34 (581)–PE–Cy7, and Fc ϵ RI (AER-37)–PE. The PBMCs were stained with the antibody mix for 30 min on ice in the dark, and then washed twice in FACS buffer before flow cytometry analysis in an LSR Fortessa cytometer (BD Biosciences). The data analysis was performed using FlowJo software version 10.

2.5 | Birch pollen-specific IgE and blood eosinophils

Serum was collected after centrifugation of coagulated blood samples. The serum was kept at -80°C before analysis. The levels of birch pollen-specific IgE in serum was measured using the ImmunoCAP system (Immunodiagnosics, Thermo Fisher Scientific). Blood eosinophils counts were performed using a Beckman Coulter HMX analyzer (Beckman Coulter, Fullerton).

2.6 | Statistical analyses

A value of $p < .05$ was considered statistically significant. Ordinal data such as asthma symptoms (score from 0 to 3), ACQ and ACT scores, and other non-parametric paired data were analyzed using Wilcoxon signed rank test. Unpaired or paired Student's *t*-test was used when comparing two groups and correlation

analyses were determined by Spearman's rank correlation test. Fisher's exact test was used to determine statistical difference of medication use.

Multivariable analysis was performed with multiple linear regression with MCp frequency as the dependent variable and sex and BMI as potential confounders. The analyses were performed with GraphPad Prism version 9.0 (GraphPad Software Inc.) and Stata version 17 (College Station).

3 | RESULTS

3.1 | Research participants characteristics

The research participants consisted of 16 women and 17 men with an asthma diagnosis. All patients had positive birch pollen specific IgE. When asked, 48% also reported sensitivity to pollen or grass, 27% to domestic pets, and 15% to house dust mite. The demographic characteristics of all the 33 adult patients are summarized in Table 1. The

FEV₁ (% of predicted), FEV₁/FVC ratio, FeNO, blood eosinophils, and birch pollen specific IgE values did not differ between birch pollen season (May) and out of season (November) (Table 2). However, a minor decrease in PEF (% of predicted) was observed during November ($p < .006$). Fewer patients reported taking oral antihistamines (24%) and inhaled corticosteroid (27%) in November, whereas 91% and 73% of the research participants were taking the respective treatments in May (Table 2).

3.2 | Higher frequency of circulating MCps during pollen season than out of season

Blood sampled in May and November were analyzed for blood circulating MCps, identified as CD4⁺ CD8⁻ CD19⁻ CD14⁻ CD34^{hi} CD117⁺ FcεRI⁺ cells by flow cytometry (Figure 1A, Figure S1A). We did not find a correlation between MCp frequency and age, BMI, birch pollen-specific IgE, blood eosinophils, or lung function indices (Table 3). However, there was a positive correlation between

General characteristics	
Age (years)	48 ± 10 (23–65)
Height (cm)	175 ± 11 (155–197)
Weight (kg)	81 ± 17 (54–123)
BMI (kg/m ²)	Normal (18.5–24.99): 13 Overweight (25–29.99): 16 Obese (>=30): 4
Sex	16 women 17 men
Positive birch pollen specific IgE (% of patients)	100%
Other self-reported sensitivity	Other pollen/grass: 48% Domestic pets: 27% House dust mite: 15%

TABLE 1 General characteristics of the 33 included research participants.

Note: The data is displayed as mean ± SD (minimum-maximum value). For BMI: the range of BMI determined by WHO to characterize normal, overweight, and obese subjects, and the number of research subjects that belonged to each BMI category.

	Pollen season May	Out of season November	p value
FEV ₁ % of predicted	86.6 ± 14.9 (43–131)	85.8 ± 15.2 (38–128)	.84
PEF % of predicted	91.4 ± 22 (47–129)	86.9 ± 20.1 (50–130)	.006
FEV ₁ /FVC ratio	75.1 ± 7 (61–86)	74.4 ± 7.5 (49–85)	.35
FeNO (ppb)	27.9 ± 21 (5–101)	25.1 ± 13.9 (5–58)	.68
Blood eosinophils (cell × 10 ³)	0.18 ± 0.12 (0.03–0.5)	0.17 ± 0.12 (0.04–0.5)	.78
Birch pollen-specific IgE (kE/L)	9.5 (5.4–16)	9.6 (5.5–17)	.75
Oral antihistamine use	91%	24%	<.0001
Inhaled corticosteroids use	73%	27%	.0005

TABLE 2 Study participants characteristics by group.

Note: The data is displayed as mean ± SD (minimum-maximum value). Birch pollen-specific IgE values are shown as geometric mean (95% CI). Statistical analysis was performed using Wilcoxon signed rank test, except for medication where Fisher's exact test was used.

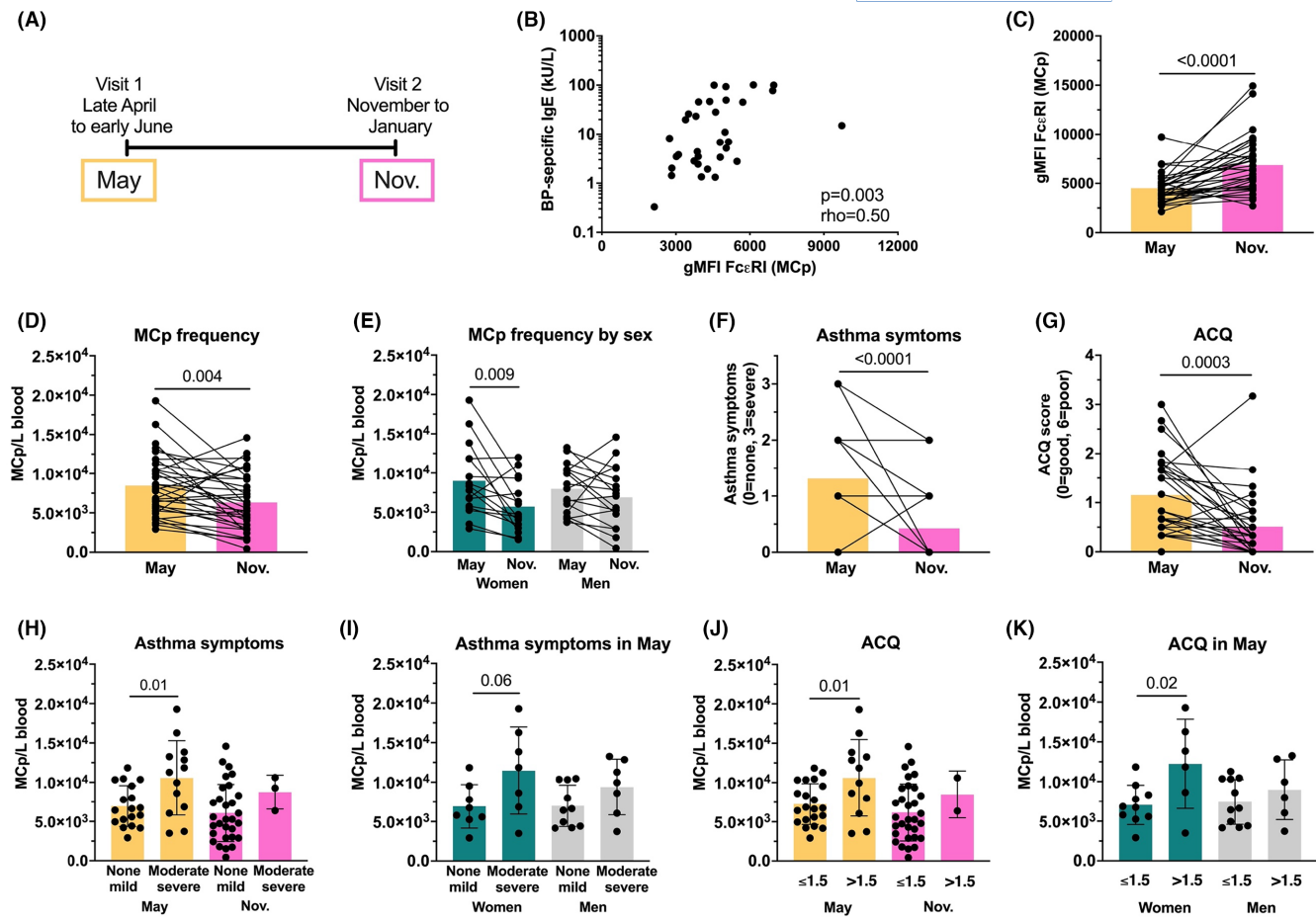


FIGURE 1 The frequency of circulating MCp is increased during birch pollen season. (A) Peripheral blood samples and symptoms surveys were taken during birch pollen season (May) and out of the season in November. (B) Spearman's correlation analysis between birch pollen (BP)-specific IgE and the geometric mean fluorescence intensity (gMFI) for Fc ϵ RI by MCps in May. (C,D) Paired analyses of the gMFI for Fc ϵ RI by MCps (C), and the frequency of circulating MCps in May and November. (E) Comparison of the MCp frequency in women and men during May and November. (F,G) Asthma symptoms (none, mild, moderate, and severe) and ACQ assessed in May and November. (H,I) MCp frequency in patients that report none/mild, or moderate/severe asthma symptoms during May and November. (H), and dichotomized by sex during May (I). (J,K) MCp frequency dichotomized by the median ACQ in May (1.5) shown during May and November (J), and dichotomized by sex during May (K). Bars represent means \pm SD. Statistical analysis was performed by Wilcoxon signed test for paired samples in C-G, and by unpaired Student's *t*-test in H-K.

the Fc ϵ RI surface expression in MCps and birch pollen-specific IgE in May (Figure 1B), but not in November ($p = .2$; $r = 0.24$). Still, the Fc ϵ RI-signal in MCps was higher in November than in May (Figure 1C), in both women and men (Figure S1B). This was not unique to the MCp population, also other CD34⁻ CD117^{-/low} Fc ϵ RI⁺ blood cells showed a higher Fc ϵ RI-signal in November (Figure S1C). Overall, the MCp frequency was higher in May compared to November (Figure 1D). This relationship was true for 25 patients, whereas 8 (3 women and 5 men) showed the opposite relationship, that is, higher MCp frequency in November than in May. Interestingly, while these 8 patients had a similar MCp frequency, asthma symptoms and asthma control as the other patients in May, they had twice the frequency of MCp as the other patients in November (Figure S1D). When all 33 analyzed patients were divided by sex and season, the higher MCp frequency in May was only significant in women (Figure 1E).

3.3 | Higher MCp frequency during pollen season in patients with more asthma symptoms and higher ACQ scores

Most patients reported more asthma symptoms and reduced asthma control (high ACQ scores) in pollen season. In May, 14 patients reported moderate to severe asthma symptoms, 11 patients reported mild symptoms, and 7 had no asthma symptoms (Figure 1F). However, in November, none of the patients reported severe asthma symptoms, only 3 patients reported moderate asthma symptoms, 8 patients had mild asthma symptoms, and 22 no asthma symptoms. The ACQ score, which assess asthma control the last week before the visit, was on average higher in May than in November (1.2 vs. 0.5; Figure 1G). This relationship was true for 25 patients, whereas 4 showed the opposite, that is, higher ACQ in November than in May, and 4 had the same score. When the MCp data was dichotomized

	Pollen season May		Out of season November	
	p value	rho	p value	rho
Asthma symptoms	.06	0.31	.53	0.11
ACQ score	.08	0.30	.67	-0.07
Age	.46	-0.13	.23	-0.21
BMI	.97	-0.005	.38	0.15
FEV ₁ % of predicted	.86	0.03	.52	0.11
PEF % of predicted	.91	-0.01	.18	0.23
FEV ₁ /FVC ratio	.30	0.18	.17	0.24
FeNO	.21	0.22	.41	0.14
Blood eosinophils	.91	0.01	.28	0.19
BP-specific IgE	.30	0.18	.45	-0.13
Δ FEV ₁ %	.87	-0.02	.76	-0.05
Δ PEF%	.89	-0.02	.36	-0.16
MCps/L blood Women versus Men	.45	-	.35	-

Note: Δ calculated as percentage of the predicted value change before and after bronchodilator. A possible correlation was tested using Spearman's analysis. Differences in MCps/L blood between Women and Men that was tested using unpaired Student's *t*-test.

Abbreviation: BP, Birch pollen.

TABLE 3 Analyses between MCp frequency and different parameters.

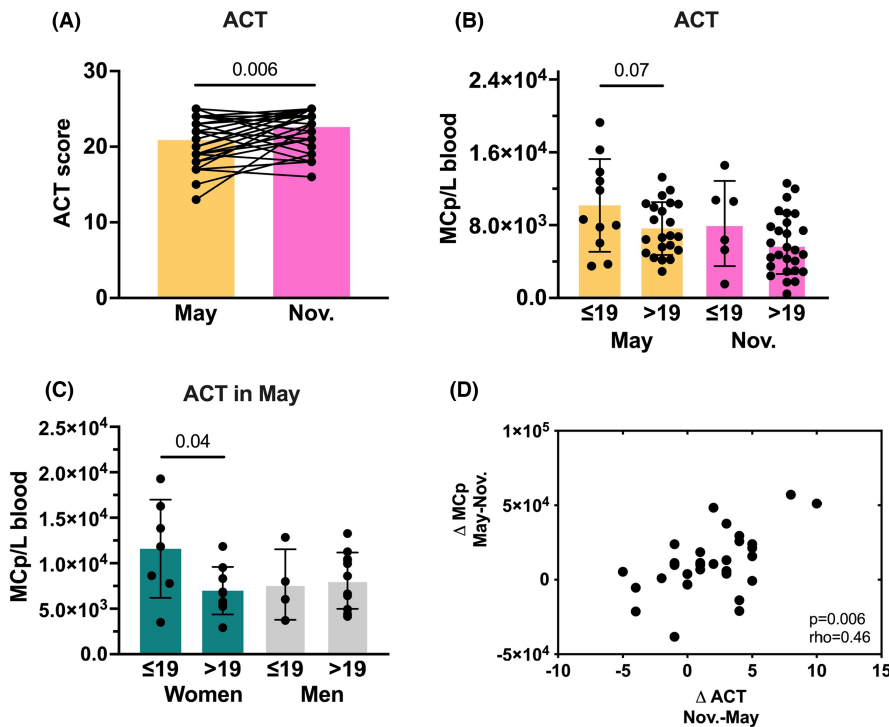
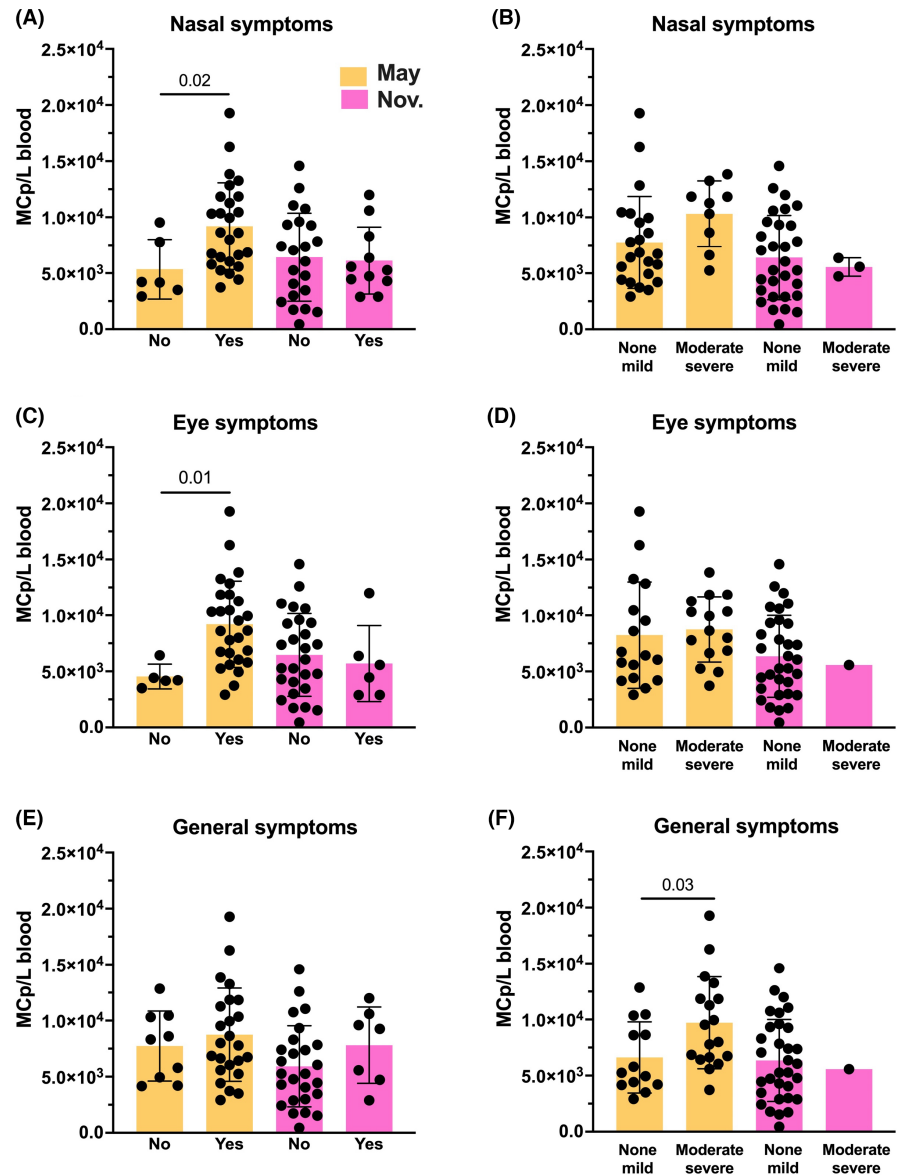


FIGURE 2 An increase in MCp frequency in May correlates with a decrease in asthma control. (A) ACT scores assessed in May and November (B) MCp frequency in patients that reported reduced (≤ 19) or better (> 20) asthma control in May (yellow) and November (pink). (C) The frequency of MCps in women and men divided by their ACT scores during May. (D) Correlation analysis between delta MCp frequency between May and November, and delta ACT between November and May. Bars represent means \pm SD. Statistical analysis was performed by Wilcoxon signed test for paired samples in A, and by unpaired Student's *t*-test in B, C. Spearman's correlation analysis was performed in D.

by the severity of asthma symptoms, patients that reported severe to moderate asthma symptoms in May had higher MCp frequency than those reporting none or mild asthma symptoms (Figure 1H). Furthermore, when asthma symptom severity in May were divided by sex, women with moderate to severe asthma symptoms tended to have higher MCp frequency than those with no or mild asthma

symptoms ($p = .06$; Figure 1I). Next, the patients were dichotomized using 1.5 as ACQ cut off according to the literature.²³ Patients reporting an ACQ score > 1.5 , reflecting reduced asthma control the last week, had a higher MCp frequency than those having ACQ ≤ 1.5 in May (Figure 1J). Out of pollen season, only two patients reported ACQ > 1.5 . When the patients were divided by sex, women with

FIGURE 3 The MCp frequency is higher in patients with allergy symptoms in May. The MCp frequency in patients that report or lack nasal (A), eye (C), or general symptoms (E), and dichotomized according to the severity of the respective symptoms (B, D, F) during May (yellow) and November (pink). Bars represent mean \pm SD. Statistical analysis was performed by unpaired Student's *t*-test.



ACQ > 1.5 had a higher MCp frequency than women with ACQ \leq 1.5 in May (Figure 1K).

3.4 | A change to higher MCp frequency correlates with a change to lower ACT

The ACT score, which reflects asthma control in the past 4 weeks, was on average lower in May than in November, that is, indicating reduced asthma control (Figure 2A). Patients that reported ACT scores < 20 during May tended to have an increased MCp frequency (Figure 2B), and when the patients were dichotomized by sex, this difference was only found in women (Figure 2C). Furthermore, the relationship between the changes in MCp frequency (delta MCp) and change in ACT (delta ACT) between November and May was analyzed among all subjects. Interestingly, the delta MCp frequency (May–November) correlated positively with delta ACT (November–May) (Figure 2D).

3.5 | Higher MCp frequency is associated with seasonal allergy symptoms

In our cohort, 40% of the asthma patients reported rhinitis in May, and there was a trend for these patients to have a higher MCp frequency than those that did not report rhinitis ($p = .053$; Figure S1E). To investigate whether other symptoms than asthma could be related with MCp frequency, the presence and absence of nasal, eye, or general symptoms, and these symptoms dichotomized by severity were recorded, and tested for a possible relation with MCp frequency. Patients with nasal and eye symptoms during May displayed higher MCp frequency regardless of the symptom severity (Figure 3A–D). However, there was a tendency for higher MCp frequency in patients that did not report skin symptoms compared to those that reported skin symptoms ($p = .07$), and there was no relation to skin symptom severity ($p = .82$). There were no differences in MCp frequency between patients that reported general symptoms such as fatigue, difficulty concentrating, or reduced physical activity

compared to those did not report such symptoms (Figure 3E). Still, the MCp frequency was higher in the group of patients that reported moderate to severe general symptoms than in those that reported no or mild symptoms during May (Figure 3F).

3.6 | Multivariable analyses

The association between MCp frequency and asthma symptoms (non and mild vs. moderate and severe) and ACQ (≤ 1.5 vs. > 1.5) during the pollen season remained significant after adjusting for sex and BMI ($p = .011$ and $.020$, respectively). The same was true for the association between MCp frequency and the presence of nasal and eye symptoms during the pollen season ($p = .020$ and $.023$, respectively).

4 | DISCUSSION

Here, we demonstrated that the frequency of blood circulating MCps is increased during birch pollen season in birch pollen-sensitized asthma patients. The increased MCp frequency was related to a decrease in asthma control. Thus, the level of circulating MCps in allergic asthma appears to reflect a changing disease pattern presumably relating to allergen exposure.

As expected, birch pollen-sensitized asthma patients in our study had more asthma symptoms, higher ACQ, and lower ACT during birch pollen season. Further analysis revealed that patients that reported moderate to severe asthma symptoms, or reduced asthma control according to the ACT and ACQ scores had the highest MCp frequency during birch pollen season. This is intriguing as MC numbers seem to rise in tissues affected by allergic symptoms suggesting either active MCp recruitment or local expansion of MCs at these sites in response to allergen exposure. For example, a bronchial allergen challenge induced increased MC numbers in the bronchial epithelium and submucosa 1 day after the challenge in patients with atopic asthma.¹³ Similarly, the total number of MCs in the nasal mucous membrane was increased during pollen season compared to out of season in patients with allergic rhinitis,¹⁴ and natural pollen exposure in patients with allergic conjunctivitis induced an MC increase in the lamina propria of the conjunctiva, where MCs normally were absent.²⁴ This is in line with earlier studies using experimental mouse models of allergic airway inflammation, which demonstrated that allergen exposure indeed led to an increase in mature MCs at specific sites, such as in the airway epithelium, and close to smooth muscle.^{15,18,25,26} Strikingly, the increase in lung MCs in these experimental systems was preceded by MCp recruitment from the bone marrow, via the blood, into the lung, where mature MCs appeared after the peak of lung MCps, which occurred during acute inflammation. Thus, we favor the view that the increased MCp frequency in the blood during May in the birch pollen-sensitized asthma patients is due to allergen-induced mobilization of bone marrow-derived MCps,

which become directed to move to specific sites of the affected organs and mature.

Despite the frequent use of medication in pollen season, more patients reported eye and nose symptoms, and moderate to severe asthma symptoms during this period. Remarkably, these asthma/allergy-related deteriorations were associated to higher frequency of circulating MCps. An increase in allergy-related symptoms during pollen season is well documented,^{3,4} and allergic asthma and rhinitis are diseases that commonly coexist. In fact, the presence of allergic rhinitis is a risk factor for asthma development.²⁷ In our study, the 40% asthma patients that had a rhinitis diagnosis showed a trend for having a higher MCp frequency in May. Thus, the symptoms reported by the patients in our study can be partially attributed to a rhinitis component.

In the present study, we did not find differences in FEV₁ or the FEV₁/FVC ratio, blood eosinophils, or other markers of lung inflammation between in and out of birch pollen season. In line with these data, an earlier study reported no changes in blood eosinophils or spirometry parameters between in and out of pollen season.²⁸ Still, a small reduction in PEF values was observed out of season in the present study. We speculate that this might reflect that fewer patients were using inhaled corticosteroids and oral antihistamines out of season. In the present study, the MCp frequency did not correlate to any lung function parameter, neither in nor out of pollen season. In contrast, our previous study of a cohort with young well-controlled patients with allergic asthma showed a higher MCp frequency in patients with reduced lung function (FEV₁ and PEF [% of predicted]).²⁰ These differences might be explained by differences between the cohorts; in the present study, a smaller and older patient population (mean age 48 years) were investigated compared to Salomonsson et al., (mean age 25 years). Both cohorts displayed relatively good lung function with FEV₁ 90% of predicted (median) in²⁰ versus FEV₁ 85% of predicted in the present cohort.

In a previous study, we demonstrated that MCps can be activated by IgE-mediated crosslinking of FcεRI.²⁹ In the present study, FcεRI expression in MCps correlated positively with serum birch-pollen-specific IgE levels in May (but not in November). This might be expected since previous studies have demonstrated that increased total IgE levels correlate with FcεRI expression in basophils from atopic and non-atopic individuals.^{30,31} Moreover, when IgE is targeted by the anti-IgE antibody omalizumab, which lowers IgE levels, this treatment also lowers FcεRI expression in MCs and basophils,^{32,33} suggesting a close relationship between FcεRI expression and total IgE. However, when we compared the FcεRI surface signal in MCps between seasons, the signal of FcεRI was higher in November than in May. Similarly, a previous report showed higher FcεRI expression in basophils out of pollen season and decreased IgE-binding in FcεRI⁺ basophils during pollen season.³⁴ Downregulation of the FcεRI-signal can be due to receptor cleavage, internalization, or suppressing signals decreasing expression. However, another possibility is steric hindrance due to

allergen bound to the specific IgE (on the FcεRI) preventing anti-FcεRI antibodies to bind to their target in the flow cytometry analysis. Further studies are needed to clarify the mechanism behind the observed reduction in FcεRI-signal in MCps during allergen exposure.

Sex dysmorphism in asthma is widely reported. For example, the prevalence of asthma is higher in adult women, but before puberty, asthma is more prevalent in boys.^{35,36} In our previous study,²⁰ women with allergic asthma had a higher MCp frequency than men, but no sex difference in MCp frequency was found among the healthy controls.²⁰ In the present study, we did not find a general difference in MCp frequency between men and women, neither in birch pollen season nor out of season. Still women displayed significantly higher circulating frequency of MCp in birch pollen season compared to out of the season, and the women with reduced asthma control had the highest MCp frequency. These patterns were weaker among men in our cohort, possibly due to that almost all men had good asthma control and no or mild asthma symptoms even in May. The mechanisms driving general sex differences in asthma are an area of extensive study. A recent meta-analysis of sex-specific pathways showed enrichment for IL-17 and chemokine signaling pathways in women but not in men,³⁷ suggesting that directed migration of immune cells might be differently regulated in women and men with asthma. However, more studies are needed to investigate these findings.

One limitation of our study is the lack of information on pollen exposure of the patients that were sampled in pollen seasons over four different years. Moreover, the sampling of the patients occurred both at the beginning and closer to the end of the birch pollen season, which may have contributed to the variation of asthma control and symptoms reported by the patients during this period. Furthermore, we could not quantify tissue MCs due to the lack of bronchial biopsies from the patients. Future studies will reveal whether increased MC numbers in airway epithelium are occurring in the same asthma patients who have had an (earlier) increased frequency of circulating MCps.

To conclude, our results indicate that the MCp frequency is higher in patients with allergic asthma that have reduced asthma control, and moderate to severe asthma and allergy symptoms during the birch pollen season. We speculate that in symptomatic patients, allergen exposure impacts the bone marrow to produce more MCps, which during active recruitment to the airways, leads to a transient increase of MCps in the bloodstream. Out of season, the allergen exposure is not present anymore, and thus the MCp frequency in the blood declines. These findings might also suggest that MCs accumulate in the airways in connection with symptom onset during natural allergen exposure. Understanding the relationships between cellular components and clinical symptoms is crucial to improve therapeutic approaches aimed at controlling asthma.

AUTHOR CONTRIBUTIONS

Christer Janson, Sara Wernersson, Andrei Malinovschi, and Jenny Hallgren conceived and designed the study. P. Abigail Alvarado-Vazquez, Erika Mendez-Enriquez, and Maya Salomonsson prepared the samples and performed the flow cytometry. P. Abigail

Alvarado-Vazquez performed the data analysis and the statistical analysis. Christer Janson performed the multivariate analysis. P. Abigail Alvarado-Vazquez, Andrei Malinovschi, Christer Janson, and Jenny Hallgren interpreted the data. P. Abigail Alvarado-Vazquez and Jenny Hallgren wrote the paper. All authors critically assessed and approved the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Pavord ID, Beasley R, Agustí A, et al. After asthma: redefining airways diseases. *Lancet*. 2018;391(10118):350-400.
2. Gallucci M, Carbonara P, Pacilli AMG, di Palma E, Ricci G, Nava S. Use of symptoms scores, spirometry, and other pulmonary function testing for asthma monitoring. *Front Pediatr*. 2019;7:54.
3. Kitinoja MA, Hugg TT, Siddika N, Rodriguez Yanez D, Jaakkola MS, Jaakkola JJK. Short-term exposure to pollen and the risk of allergic and asthmatic manifestations: a systematic review and meta-analysis. *BMJ Open*. 2020;10(1):e029069.
4. Carlsen HK, Haga SL, Olsson D, et al. Birch pollen, air pollution and their interactive effects on airway symptoms and peak expiratory flow in allergic asthma during pollen season - a panel study in northern and southern Sweden. *Environ Health*. 2022;21(1):63.
5. Eriksson J, Bjerg A, Lötvalld J, et al. Rhinitis phenotypes correlate with different symptom presentation and risk factor patterns of asthma. *Respir Med*. 2011;105(11):1611-1621.
6. D'Amato G, Cecchi L, Bonini S, et al. Allergenic pollen and pollen allergy in Europe. *Allergy*. 2007;62(9):976-990.

7. Biedermann T, Winther L, Till SJ, Panzner P, Knulst A, Valovirta E. Birch pollen allergy in Europe. *Allergy*. 2019;74(7):1237-1248.
8. Mendez-Enriquez E, Hallgren J. Mast cells and their progenitors in allergic asthma. *Front Immunol*. 2019;10:821.
9. Amin K, Janson C, Boman G, Venge P. The extracellular deposition of mast cell products is increased in hypertrophic airways smooth muscles in allergic asthma but not in nonallergic asthma. *Allergy*. 2005;60(10):1241-1247.
10. Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast-cell infiltration of airway smooth muscle in asthma. *N Engl J Med*. 2002;346(22):1699-1705.
11. Dougherty RH, Sidhu SS, Raman K, et al. Accumulation of intraepithelial mast cells with a unique protease phenotype in T(H)2-high asthma. *J Allergy Clin Immunol*. 2010;125(5):1046-1053 e1048.
12. Altman MC, Lai Y, Nolin JD, et al. Airway epithelium-shifted mast cell infiltration regulates asthmatic inflammation via IL-33 signaling. *J Clin Invest*. 2019;129(11):4979-4991.
13. Wang W, Li Y, Lv Z, et al. Bronchial allergen challenge of patients with atopic asthma triggers an Alarmin (IL-33, TSLP, and IL-25) response in the airways epithelium and submucosa. *J Immunol*. 2018;201(8):2221-2231.
14. Viegas M, Gomez E, Brooks J, Davies RJ. Changes in nasal mast cell numbers in and out of the pollen season. *Int Arch Allergy Appl Immunol*. 1987;82(3-4):275-276.
15. Abonia JP, Hallgren J, Jones T, et al. Alpha-4 integrins and VCAM-1, but not MAdCAM-1, are essential for recruitment of mast cell progenitors to the inflamed lung. *Blood*. 2006;108(5):1588-1594.
16. Xing W, Austen KF, Gurish MF, Jones TG. Protease phenotype of constitutive connective tissue and of induced mucosal mast cells in mice is regulated by the tissue. *Proc Natl Acad Sci U S A*. 2011;108(34):14210-14215.
17. Bankova LG, Dwyer DF, Liu AY, Austen KF, Gurish MF. Maturation of mast cell progenitors to mucosal mast cells during allergic pulmonary inflammation in mice. *Mucosal Immunol*. 2015;8(3):596-606.
18. Mendez-Enriquez E, Alvarado-Vazquez PA, Abma W, et al. Mast cell-derived serotonin enhances methacholine-induced airway hyperresponsiveness in house dust mite-induced experimental asthma. *Allergy*. 2021;76(7):2057-2069.
19. Dahlin JS, Malinovsky A, Ohrvik H, et al. Lin⁻ CD34^{hi} CD117^{int}/hi FcepsilonRI⁺ cells in human blood constitute a rare population of mast cell progenitors. *Blood*. 2016;127(4):383-391.
20. Salomonsson M, Malinovsky A, Kalm-Stephens P, et al. Circulating mast cell progenitors correlate with reduced lung function in allergic asthma. *Clin Exp Allergy*. 2019;49(6):874-882.
21. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J*. 2005;26(2):319-338.
22. American Thoracic Society, European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med*. 2005;171(8):912-930.
23. Juniper EF, Bousquet J, Abetz L, Bateman ED, Committee G. Identifying 'well-controlled' and 'not well-controlled' asthma using the asthma control questionnaire. *Respir Med*. 2006;100(4):616-621.
24. Anderson DF, MacLeod JD, Baddeley SM, et al. Seasonal allergic conjunctivitis is accompanied by increased mast cell numbers in the absence of leucocyte infiltration. *Clin Exp Allergy*. 1997;27(9):1060-1066.
25. Hallgren J, Jones TG, Abonia JP, et al. Pulmonary CXCR2 regulates VCAM-1 and antigen-induced recruitment of mast cell progenitors. *Proc Natl Acad Sci U S A*. 2007;104(51):20478-20483.
26. Dahlin JS, Feinstein R, Cui Y, Heyman B, Hallgren J. CD11c⁺ cells are required for antigen-induced increase of mast cells in the lung. *J Immunol*. 2012;189(8):3869-3877.
27. Tohidinik HR, Mallah N, Takkouche B. History of allergic rhinitis and risk of asthma; a systematic review and meta-analysis. *World Allergy Organ J*. 2019;12(10):100069.
28. Kampe M, Stalenheim G, Janson C, Stolt I, Carlson M. Systemic and local eosinophil inflammation during the birch pollen season in allergic patients with predominant rhinitis or asthma. *Clin Mol Allergy*. 2007;5:4.
29. Mendez-Enriquez E, Salomonsson M, Eriksson J, et al. IgE cross-linking induces activation of human and mouse mast cell progenitors. *J Allergy Clin Immunol*. 2022;149(4):1458-1463.
30. Saini SS, Klion AD, Holland SM, Hamilton RG, Bochner BS, Macglashan DW. The relationship between serum IgE and surface levels of FcepsilonR on human leukocytes in various diseases: correlation of expression with FcepsilonRI on basophils but not on monocytes or eosinophils. *J Allergy Clin Immunol*. 2000;106(3):514-520.
31. Malveaux FJ, Conroy MC, Adkinson NF, Lichtenstein LM. IgE receptors on human basophils. Relationship to serum IgE concentration. *J Clin Invest*. 1978;62(1):176-181.
32. Saini SS, MacGlashan DW, Sterbinsky SA, et al. Down-regulation of human basophil IgE and FC epsilon RI alpha surface densities and mediator release by anti-IgE-infusions is reversible in vitro and in vivo. *J Immunol*. 1999;162(9):5624-5630.
33. Gomez G, Jogie-Brahim S, Shima M, Schwartz LB. Omalizumab reverses the phenotypic and functional effects of IgE-enhanced Fc epsilon RI on human skin mast cells. *J Immunol*. 2007;179(2):1353-1361.
34. Carlsson M, Thorell L, Sjölander A, Larsson-Faria S. Variability of total and free IgE levels and IgE receptor expression in allergic subjects in and out of pollen season. *Scand J Immunol*. 2015;81(4):240-248.
35. Postma DS. Gender differences in asthma development and progression. *Genet Med*. 2007;4 Suppl B:S133-S146.
36. Chowdhury NU, Guntur VP, Newcomb DC, Wechsler ME. Sex and gender in asthma. *Eur Respir Rev*. 2021;30(162):210067.
37. Gautam Y, Afanador Y, Abebe T, López JE, Mersha TB. Genome-wide analysis revealed sex-specific gene expression in asthmatics. *Hum Mol Genet*. 2019;28(15):2600-2614.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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