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Circulating mast cell progenitors correlate with reduced lung function in allergic asthma

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Summary

Background: Studies using mouse models have revealed that mast cell progenitors are recruited from the blood circulation to the lung during acute allergic airway inflammation. The discovery of a corresponding human mast cell progenitor population in the blood has enabled to study the relation of circulating mast cell progenitors in clinical settings.

Objectives: To explore the possible association between the frequency of mast cell progenitors in the blood circulation and allergic asthma, we assessed the relation of this recently identified cell population with asthma outcomes and inflammatory mediators in allergic asthmatic patients and controls.

Methods: Blood samples were obtained, and spirometry was performed on 38 well-controlled allergic asthmatic patients and 29 controls. The frequency of blood mast cell progenitors, total serum IgE and 180 inflammation- and immune-related plasma proteins were quantified.

Results: Allergic asthmatic patients and controls had a similar mean frequency of blood mast cell progenitors, but the frequency was higher in allergic asthmatic patients with reduced FEV_1 and PEF (% of predicted) as well as in women. The level of fibroblast growth factor 21 (FGF-21) correlated positively with the frequency of mast cell progenitors, independent of age and gender, and negatively with lung function. The expression of FcεRI on mast cell progenitors was higher in allergic asthmatic patients and correlated positively with the level of total IgE in the controls but not in the asthmatic patients.

Conclusion: Elevated levels of circulating mast cell progenitors are related to reduced lung function, female gender and high levels of FGF-21 in young adults with allergic asthma.

KEYWORDS
allergic asthma, asthma, lung function, mast cell progenitors, mast cells

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1 | INTRODUCTION

Mast cells play a significant role in asthma through their activation and production of mediators with pro-inflammatory and airway constrictive effects,[1] and perhaps even more so in severe[2] or uncontrolled asthma.[3] Asthmatic patients have an increased number of mast cells at particular sites of the lung such as in the airway smooth muscle,[4] in the bronchial epithelium[5] and in the alveolar parenchyma.[3] Haematopoietic stem cells and progenitors in the bone marrow can give rise to tissue-resident mast cells.[6] Human mast cells have been derived in vitro from peripheral or cord blood for many years.[7] However, we recently identified a population of mast cell progenitors (MCp) in the blood circulation as $CD4^-$ $CD8^-$ $CD19^-$ $CD14^-$ $CD34^{hi}$ $CD117^+$ $Fc\varepsilon RI^+$ cells, thus allowing the quantification and characterization of this rare population in healthy and diseased individuals.[8]

Previous studies in mice using a sensitization and challenge model addressed the mechanism behind the expansion of lung mast cells in asthma. The antigen-induced inflammation caused a strong influx of bone marrow-derived MCp from the blood to the lung, which was inhibited by antibodies blocking alpha 4 integrins and VCAM-1, or genetic deletion of endothelial VCAM-1.[9] At later timepoints after the induction of allergic lung inflammation, an increased number of mast cells were demonstrated in the tracheal epithelium[10] and in the alveolar parenchyma.[11] Therefore, the increase in lung mast cells in asthmatic patients is likely due to the recruitment of circulating MCp to the lung followed by in situ maturation into mast cells. In our view, an active recruitment of MCp from the bone marrow to the lung via the blood should be reflected in an increased frequency of blood MCp.

In the present study, our aim was to explore the possible relationship between MCp frequency and asthma outcomes as well as both established and novel markers of inflammation in a group of young adults with allergic asthma and controls.

2 | MATERIALS AND METHODS

2.1 | Subjects

Blood samples were obtained from 67 individuals (38 subjects with allergic asthma and 29 controls), aged 15-41 years, in a follow-up study of the MIDAS cohort (Minimally Invasive Diagnostic Procedures in Allergy, Asthma, or Food Hypersensitivity Study). The inclusion criteria for asthmatic patients in MIDAS were physician-diagnosed asthma and daily treatment with an inhaled corticosteroid (ICS) and/or an oral leukotriene receptor antagonist (LTRA) during at least three of the past 12 months before the first MIDAS.[12] The control subjects were considered non-atopic (<0.35 kU_A/L in Phadiatop and fx5; ImmunoCAP) when tested 3-5 years prior to the present study, and did not have an asthma diagnosis. In an interim analysis, the MCp frequency and the percent predicted forced expiratory volume in 1 second (FEV_1) of a few of the subjects (13 allergic asthmatic patients and 10 controls) were reported previously.[8]

2.2 | Asthma outcomes

The Asthma Control Test (ACT) was used to determine the degree of asthma control. Spirometry was performed according to ATS/ERS guidelines,[13] and the highest values of three acceptable measurements for FEV_1, forced vital capacity (FVC), and peak expiratory flow (PEF) were used for the analyses. Forced expiratory flow (FEF) rates at 25%, 50% and 75% of vital capacity exhaled were calculated from the curve with the highest sum of FEV_1 and FVC. For subjects less than 18 years of age (13 subjects), Solymar reference values were used,[14] whereas Hedenström reference values were used for subjects 18 years or older.[15]

The fraction of exhaled nitric oxide (FeNO) was measured according to the American Thoracic Society/European Respiratory Society recommendations[12] using a chemiluminescence analyser (NIOX Flex; Aerocrine AB, Solna, Sweden).

2.3 | Blood measurements

Total serum IgE was measured using the ImmunoCAP system (Immunodiagnostics, Thermo Fisher Scientific, Uppsala, Sweden). One negative value obtained for total IgE was omitted. Blood cells were counted at the Department of Clinical Chemistry and Pharmacology at Uppsala University Hospital using a routine method (Cell-Dyn Sapphire, Abbott, Illinois, USA) for 62 out of 67 subjects. The Inflammation and the Immune response panels from Olink Proteomics (Uppsala, Sweden) were used to quantify 180 different proteins in EDTA plasma by the proximity extension and ligation method.[16] For the complete lists of the analysed proteins, see the web pages for the Inflammation panel (www.olink.com/products/inflammation/biomarkers/) and the Immune response panel (www.olink.com/products/immune-response-panel/biomarkers/). For the Inflammation panel, samples from 61 subjects and for the Immune response panel, 63 subjects passed the sample quality control and were further analysed.

2.4 | Mononuclear cell enrichment

The blood was collected in EDTA-treated tubes (12-15 mL; BD Vacutainer, BD Bioscience, Franklin Lakes, NJ). Peripheral blood mononuclear cells (PBMC) were enriched using Ficoll-Paque Premium (ρ = 1.076 g/mL) (GE Healthcare, Little Chalfont, UK) in SepMate™-50 tubes (Stemcell Technologies, Vancouver, Canada). Platelets were removed by centrifugation (2 × 200 *g*, 10 minutes).

2.5 | Flow cytometry

To quantify human MCp, 10×10^6 enriched mononuclear cells were incubated in PBS, pH 7.4 with 2% heat-inactivated fetal calf serum (Sigma-Aldrich, St. Louis, MO) with the following

fluorescent-labelled antibodies; CD4 (RPA-T4), CD8 (RPA-T8), CD13 (WM15), CD14 (M5E2), CD19 (HIB19), CD34 (581), CD117 (104D2) and FcεRI (AER-37). The antibodies were obtained from BD Bioscience and eBioscience, San Diego, CA, USA. The flow cytometry was performed on a LSRII, LSRFortessa or a FACSAria III (BD Biosciences). Data analysis was performed using FlowJo software version 9.8.

2.6 | Data analyses

Statistical differences between groups were assessed using unpaired, two-tailed Student's *t* test or an ANOVA with Tukey's multiple comparison post hoc test. Means in the results text and tables are given as ±SEM. Associations between the number of MCp and continuous variables such as lung function were analysed with Spearman's rank correlation test. Linear regression was determined with Pearson's correlation. Multiple linear regression was used when analysing group difference after adjusting for lung function. Before the statistical analysis of the protein levels in plasma in relation to MCp/L blood, principal component analyses (PCA) and evaluation of total sample signal were used for quality control. The PCA demonstrated that two patients may be outliers, but their removal did not affect the results. All graphs were prepared using GraphPad Prism 7.0c (GraphPad Software Inc., San Diego, CA). The statistical analyses were made either using GraphPad or R (version 3.4.3; R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/). Multiple analyses were adjusted with false discovery rate analysis using the Benjamini-Hochberg method of correction for multiple testing. A *P*-value equal to or less than 0.05 was considered significant.

2.7 | Ethical statement

All subjects gave their written informed consent to participate in the study, which was approved by the Uppsala Regional Ethics Review Board (Dnr 2012/420).

3 | RESULTS

3.1 | Subject characteristics and quantification of blood mast cell progenitors

Blood from 38 allergic asthmatic patients and 29 controls of similar age, height, weight and body mass index (Table 1) was analysed for the frequency of circulating MCp by flow cytometry (Figure 1A).[8] The MCp frequency expressed as MCp/10^6 peripheral blood mononuclear cells (PBMC) correlated to the frequency of MCp/L blood (Figure 1B). The overall mean frequency of MCp was 1.07 ± 0.58 × 10^5/L blood and 71.3 ± 42.1 MCp/10^6 PBMC. Patients with allergic asthma and controls had a similar mean frequency of MCp/10^6 PBMC or MCp/L blood (Figure 1C).

3.2 | The frequency of circulating mast cell progenitors is higher in women and subjects with reduced lung function

Next, the relationship between MCp frequency, asthma outcomes and blood cell counts was analysed. No correlations could be found for analyses of MCp frequency with forced vital capacity (FVC), mean forced expiratory volume in 1 second (FEV_1)/FVC, forced expiratory flow $(FEF)_{75,}$ fraction of exhaled nitric oxide (FeNO), ACT score or any blood cell differential count. However, a higher MCp frequency correlated with reduced FEV_1 (% predicted) in allergic

TABLE 1 Subject characteristics

	Allergic asthma (n = 38)	Controls (n = 29)	*P*-value
Female gender	21 (55%)	18 (62%)	
Age (y)	25 ± 1	23 ± 1	0.31
Height (cm)	175 ± 1.8	172 ± 9.8	0.16
Weight (kg)	68.8 ± 12.7	71.2 ± 18.1	0.52
BMI	22.4 ± 4.5	22.5 ± 4.1	0.78
ACT	21 ± 2.9	NA	-
ICS	7	0	-
ICS+LABA	23	0	-
LTRA	5	0	-
Total IgE (kU/L)	441.4 ± 75.4	34.8 ± 11.8	<0.0001
Blood eosinophils (x10^9/L)	0.2	0.1	0.0002
FeNO (ppb)	24.5	11.0	<0.0001

ACT, asthma control test; BMI, body mass index; FeNO, fraction of inhaled nitric oxide; ICS, inhaled corticosteroid; ICS+LABA, inhaled corticosteroid + long-acting beta agonists; LTRA, leukotriene receptor antagonists; NA, not applicable.
The results are presented as mean± SEM except for blood eosinophils and FeNO, which are given as the median.

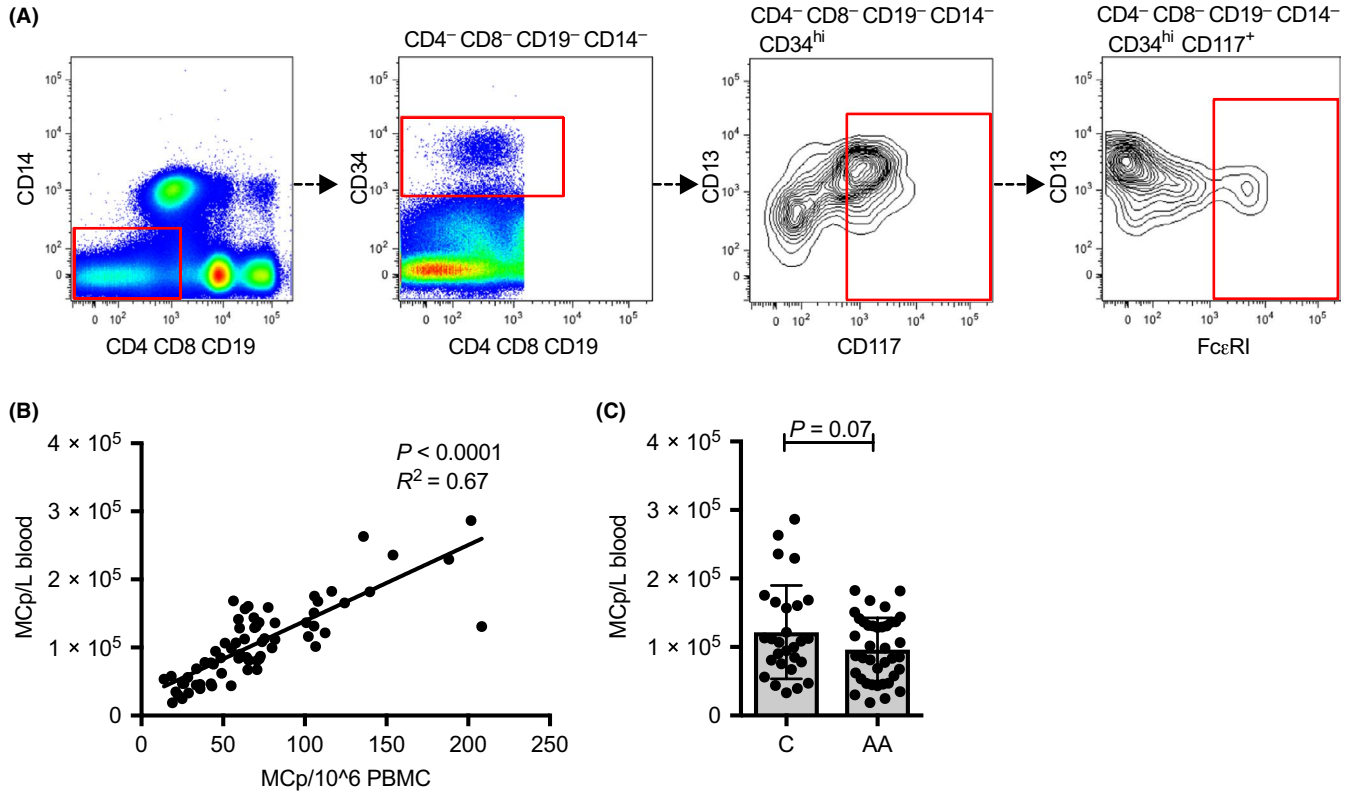

FIGURE 1 Allergic asthmatic patients and controls have a similar frequency of circulating MCp. A, The flow cytometry gating strategy for quantifying blood MCp. MCp were identified as $CD4^-$ $CD8^-$ $CD19^-$ $CD14^-$ $CD34^{hi}$ $CD117^+$ $Fc\varepsilon RI^+$ cells. B, The frequency of MCp/L blood correlates to the frequency of MCp/10^6 PBMC in all subjects. Pearson's correlation was used to determine linear regression. C, The study subjects were grouped as allergic asthmatic patients (AA) and controls (C). The overall mean±SD frequency of MCp/L blood in C and AA are shown. The difference between two groups was tested with Student's *t* test

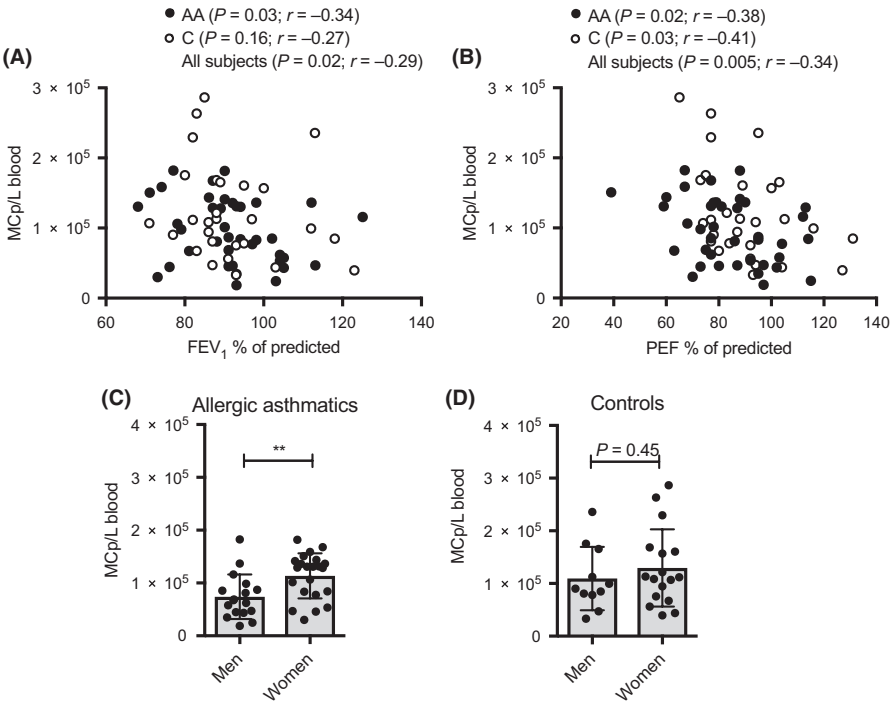

FIGURE 2 Individuals with a reduced lung function have an increased frequency of circulating MCp. (A, B) Correlation analysis of MCp/L blood with FEV_1% of predicted (A) and PEF % of predicted (B), among all subjects, allergic asthmatic patients (AA) and controls (C). A possible correlation between the MCp frequency and the different lung function parameters was tested using Spearman's correlation analysis. (C, D) The allergic asthmatic patients (C) and controls (D) were divided according to gender and the frequency of MCp compared between the groups. Means ±SD; $^*P \leq 0.05$; $^{**}P < 0.01$

asthmatic patients, but not in the controls (Figure 2A). This relation was consistent when dividing all subjects or allergic asthmatic patients into two groups according to the median FEV_1 (90% of predicted; Figure S1A,B). The analysis demonstrated that subjects with reduced lung function had a higher frequency of MCp in their blood circulation ($P = 0.001$) (Figure S1A). This association was also found among the allergic asthmatic patients ($P = 0.003$) (Figure S1B). Furthermore, MCp frequency correlated negatively with peak expiratory flow (PEF; % of predicted) in allergic asthmatic patients and controls (Figure 2B) as well as FEF (% of predicted; FEF_{50}: $P = 0.03$; $r = -0.35$; FEF_{25}: $P = 0.04$; $r = -0.45$) in the allergic asthmatic patients, but not in the controls.

When all subjects were grouped according to gender, women had a mean FEV_1 (% of predicted) of 89 ± 11, which was lower than the mean for men (95 ± 13; Figure S1C). Women also had a trend to a lower PEF (% of predicted) than men (Figure S1D). When the MCp frequency was compared between genders, women with allergic asthma had higher mean frequency of MCp/L blood than men (Figure 2C), and the same relationship was demonstrated among all subjects (Figure S1E). These differences remained significant after adjustment for FEV_1. However, in the controls, women and men had a similar mean MCp frequency (Figure 2D). Further, the association between lung function and MCp frequency within each gender was investigated. In women, there was also a trend towards a correlation between FEV_1 (% of predicted; $P = 0.08$) and MCp frequency (Figure S1F), and there was a correlation between PEF (% of predicted; $P = 0.005$) and frequency of MCp (Figure S1H). However, such correlations were not found in men (Figure S1G,I).

3.3 | The expression of FcεRI on mast cell progenitors is higher in allergic asthmatic patients and correlates with the level of total IgE in controls

The allergic asthmatic patients had ~11 times higher total IgE than the controls (Table 1). On average, the allergic asthmatic patients had a higher geometric mean fluorescence intensity (gMFI) of FcεRI on the MCp than the controls (Figure 3A). When the gMFI of the FcεRI expression on the MCp was compared to the level of total IgE, a positive correlation was found among all subject ($P = 0.002$) and the controls ($P < 0.0001$) (Figure 3B). However, such a correlation was not found in the allergic asthmatic patients ($P = 0.64$). In addition, there was no association between the level of total IgE and the frequency of circulating MCp (Figure 3C), FEV_1 (% of predicted) or gMFI of the FcεRI expression on the MCp (not shown).

3.4 | FGF-21 levels correlate positively with the frequency of circulating mast cell progenitors in allergic asthmatic patients

The concentration of 180 different inflammation- and immune-related proteins in plasma was determined using two proximity ligation assay panels. Eight proteins correlated with MCp/L blood

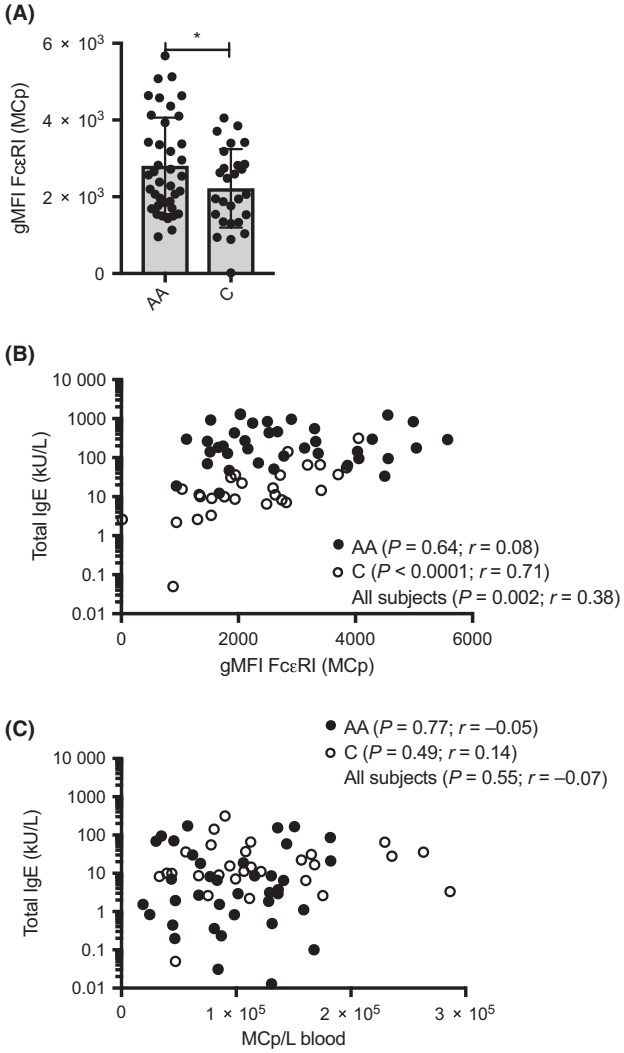

FIGURE 3 The expression of FcεRI on MCp is higher in allergic asthmatic patients and correlates with the level of total IgE in the controls. A, The geometric mean fluorescence intensity (gMFI) for the surface expression of FcεRI on the MCp was compared between allergic asthmatic patients and controls. The mean±SD is shown; $^*P \leq 0.05$. B, Spearman's correlation analysis of the surface expression of FcεRI on the MCp with the level of total IgE. In two analyses (2/67), a different fluorophore was used to determine the expression FcεRI; thus, these analyses were excluded for the calculation of gMFI. C, Correlation analysis of the frequency of MCp with the level of total IgE using Spearman's analysis

using a model of linear regression with adjustment for age and gender (Table 2). After adjustment for multiple testing (Benjamini-Hochberg), the level of fibroblast growth factor 21 (FGF-21) remained correlated with the frequency of MCp/L blood. FGF-21 also remained significant when using the more conservative Bonferroni correction for multiple testing.

Next, the subjects were divided into allergic asthmatic patients and controls, and correlation analyses for the three top hits FGF-21, and discoidin, CUB and LCCL domain–containing protein 2 (DCBLD2) and histamine N-methyltransferase (HNMT) were performed. Positive correlations between the MCp frequency and the

TABLE 2 Correlation analysis of MCp frequency and serum protein levels, including adjustment for multiple analysis using Benjamini-Hochberg method

Name	β-coefficient	*P*-value	Adjusted *P*-value	Samples over detection limit (%)
FGF-21	2.76	1.5 × 10−4	0.03	100
HNMT	0.26	0.002	0.15	79
DCBLD2	0.46	0.003	0.21	100
IL-4	−1.27	0.028	0.76	43
IFN-gamma	−0.18	0.028	0.76	63
MILR1	0.52	0.034	0.76	100
MCP-3	0.55	0.028	0.76	100
IL-5	−1.63	0.037	0.76	66

DCBLD2, discoidin, CUB and LCCL domain–containing protein 2; FGF-21, fibroblast growth factor 21; HNMT, histamine N-methyltransferase; MCP-3, monocyte chemoattractant protein-3 (CCL7); MILR1: mast cell immunoglobulin-like receptor 1.
The model for calculating correlation between MCp frequency and protein levels was adjusted for age and gender.

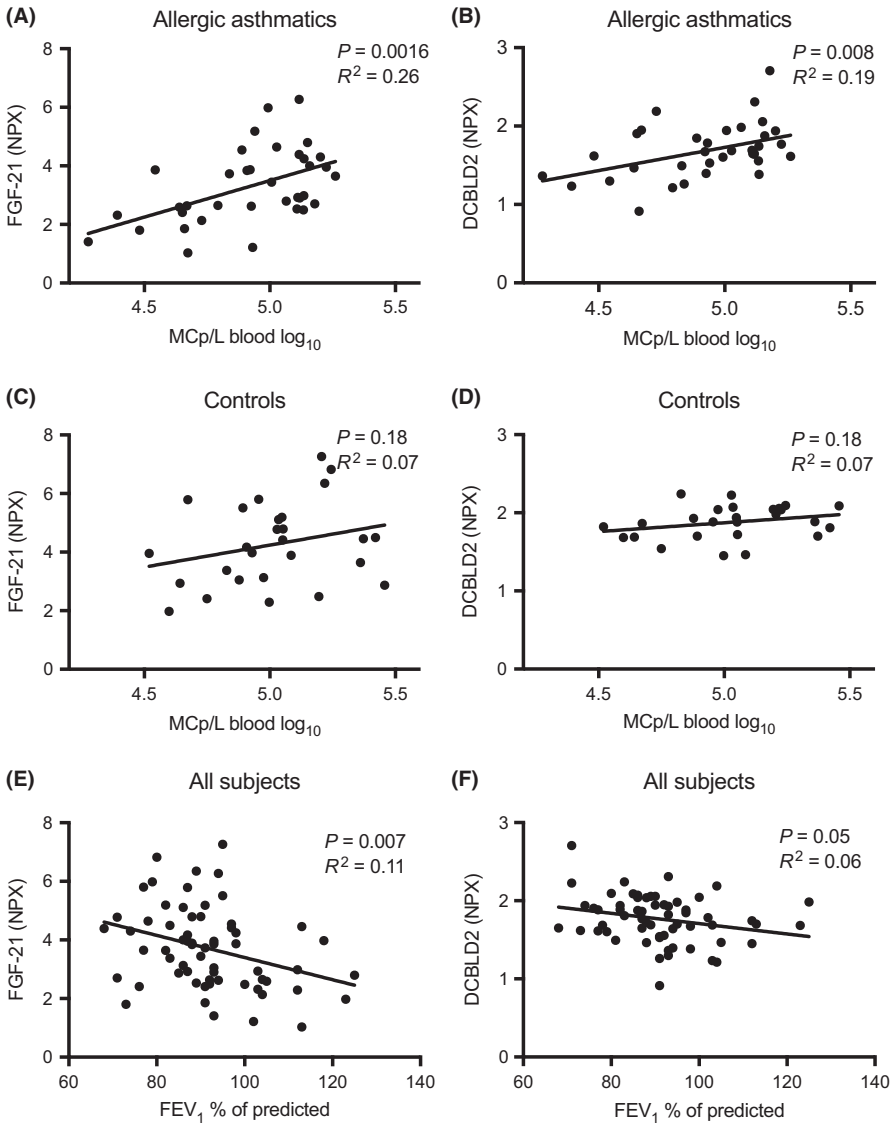

FIGURE 4 High plasma concentration of FGF-21 and DCBLD2 correlates with high frequency of MCp among allergic asthmatic patients and FEV_1 of predicted among all subjects. (A-D) The level of plasma proteins was quantified using the Inflammation and Immune response panels from Olink Proteomics. The proteins levels (normalized protein expression on a log_2 scale; NPX) and the MCp frequency (expressed as log_{10}) among allergic asthmatic patients and controls were plotted against each other. (E, F) The protein levels (normalized protein expression on a log_2 scale; NPX) of FGF-21 (E), or DCBLD2 (F), and the FEV_1 of predicted were plotted against each other. Linear regression analysis was determined with Pearson's correlation. $P \leq 0.05$ was considered significant

level of FGF-21, and DCBLD2 were found in allergic asthmatic patients, but not in the controls (Figure 4). The level of HNMT showed a trend to be positively correlated with MCp frequency in both allergic asthmatic patients and controls (Figure S2A,B). Since the MCp frequency correlated with reduced lung function, we tested whether a correlation between FGF-21, HNMT or DCBLD2 and FEV_1 (% of predicted) could be found. Both FGF-21 and DCBLD2 levels correlated negatively with FEV_1 in all subjects (Figure 4E,F) and in the controls (FGF-21, $P = 0.03$; DCBLD2, $P = 0.003$). A trend towards a negative correlation between FGF-21 and FEV_1 (% of predicted) was found in the allergic asthmatic patients ($P = 0.059$). However, no relationship was demonstrated between DCBLD2 and FEV_1 (% of predicted) in the allergic asthmatic patients ($P = 0.38$). Moreover, HNMT levels did not show any relation to FEV_1 (% of predicted) in all subjects (Figure S2C) or in allergic asthmatic patients and controls (results not shown).

4 | DISCUSSION

In this study, the frequency of circulating MCp correlated with reduced FEV_1, PEF, FEF_{25} and FEF_{50} (% predicted) among the allergic asthmatic patients, suggesting that a moderately reduced lung function among well-controlled asthmatic patients may reflect pathological changes due to mast cell infiltration. Indeed, several studies using experimental models of asthma and mice deficient in mast cells[17-19] or specific mast cell mediators, for example tryptase (mMCP-6),[20] have demonstrated that lung function is the main asthma outcome related to mast cells. MCp are recruited to the lung in similar experimental mouse models, leading to an expanded mast cell population at particular sites of the lung,[6] as also observed in asthmatic patients.[4,5] Thus, a high frequency of circulating MCp may be a sign of mast cell–driven decline in lung function.

In the present study, allergic asthmatic patients and controls had a variable but on average similar frequency of blood MCp. The explanation to this may be that the majority of the allergic asthmatic patients in the study had relatively normal lung function (the median FEV_1 was 91.5% of predicted), well-controlled asthma (mean Asthma Control Test (ACT) of 21 ± 2.9), ongoing asthma treatment and were investigated when their asthma was stable. Worth noting is that the four highest MCp frequencies detected were among the controls. When the controls were subgrouped according to their self-reported health issues, two of the four control subjects with the highest MCp frequency reported respiratory problems (none of the controls were treated with inhaled corticosteroids). We recently found that during acute influenza infection in mice, innate immunity triggers the recruitment of MCp to the lung to a similar extent as allergic inflammation.[21,22] The individuals included in our study had to be free from cold symptoms 2 weeks prior to the clinic visit. However, some may have had a prior or subclinical respiratory infection that caused the higher levels of circulating MCp, and possibly could explain a slightly reduced lung function in the controls.

As reviewed by Postma,[23] the prevalence of asthma is higher in boys during childhood, whereas after puberty the prevalence is higher in women. When the young adults in our study were grouped according to gender, a higher mean frequency of circulating MCp was found among women, and this relationship was strongest among the allergic asthmatic patients. Although adult men have larger airways, especially trachea, than women,[24] adjustment for gender is part of the calculation for FEV_1 (% of predicted), despite this there is a difference in FEV_1 between the genders in this study. Future investigations will tell whether the gender difference in MCp frequency is general or related to this cohort.

Asthma is associated with high total IgE levels, independently of the presence of IgE sensitization.[25,26] Moreover, the biology of mast cells is tightly connected to the expression of FcεRI and the binding of IgE to this receptor enhances the expression in mice.[27] To determine whether a similar relationship exists in human MCp, the gMFI of FcεRI on the MCp was compared to the level of total IgE. As expected from the higher total serum IgE found in the allergic asthmatic patients, these patients had a higher gMFI of FcεRI on the MCp than the control group. However, the level of total IgE correlated positively with FcεRI expression on the MCp among the controls, but not among the allergic asthmatic patients, which further supports that the level of IgE regulates the FcεRI expression on MCp, up to a saturation point. Nevertheless, the level of total IgE was not related to the frequency of circulating MCp. In mice, intranasal administration of IgE immune complexes increases the frequency and total number of lung MCp in antigen-sensitized mice compared to control mice given the same dose of antigen alone in a process dependent on an Fc receptor associated with the FcRγ chain, possibly FcεRI.[28] Thus, another avenue to explore would be to test whether allergic asthmatic patients acutely exposed to the allergen they are sensitized to have an increased frequency of circulating MCp during exposure compared to when they are not exposed.

In our efforts to obtain a non-biased clue to the molecular mechanisms behind having a high frequency of circulating MCp, FGF-21 was identified as the single plasma protein (out of 180) that correlated significantly with MCp frequency in all subjects after the adjustment for multiple testing. FGF-21 is a soluble protein with effects on glucose and lipid metabolism.[29] Assuming that a high MCp frequency implies recruitment of MCp to the lung, a possible connection between MCp frequency and FGF-21 is the lipid mediators leukotriene B4 and prostaglandin D2, which have been shown to attract in vitro-derived MCp to the skin of mice.[30] In subanalyses of allergic asthmatic patients and controls, the three proteins that had the lowest adjusted p-values after multiple testing were investigated. In the allergic asthmatic patients, FGF-21 and DCBLD2 were identified to positively correlate with the frequency of circulating MCp. DCBLD2 is a transmembrane protein expressed in vascular endothelial and smooth muscle cells, and associations between DCBLD2 haplotypes and a decline in FEV_1 following aspirin challenge were found in aspirin-exacerbated respiratory disease (AERD).[31] AERD is more common among women and young adults, and mast cells and lipid mediators such as cysteinyl-leukotrienes

and prostaglandin D2 are implicated in the pathogenesis.[32] Thus, the relationship between DCBLD2 levels and MCp frequency is intriguing. Worth noting is that both FGF-21 and DCBLD2 levels correlated negatively with FEV_1 in all subjects and in the controls, while in the allergic asthmatic patients, the negative correlation between FGF-21 and FEV_1 showed only a trend, and was not at all observed between DCBLD2 and FEV_1. Hence, whereas the relationship between MCp frequency and FEV_1 was significant only among the allergic asthmatic patients, FGF-21 and DCBLD2 levels were related to reduced FEV_1 in general. Further studies are needed to clarify the relationships between the MCp frequency, plasma levels of FGF-21 and DCBLD2 and FEV_1.

To conclude, a high frequency of circulating MCp correlates with reduced lung function, implying ongoing recruitment of MCp to the lung, and perhaps indicating mast cell involvement in declining lung function. The finding regarding higher levels of circulating MCp in women with allergic asthma is interesting from disease mechanism point of view, but should be replicated in other patient populations.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

JSD and JH conceived the study. KA was mainly responsible for the MIDAS cohort. MS, AM, JSD, CJ, KA and JH designed the study. PKS invited the participants and performed the clinical measurements. MS and JSD prepared the samples and performed the flow cytometry. MS performed the data analysis and the statistical analysis. AM, CJ, KA and JH interpreted and supervised the data analysis and the statistical analysis. MS and JH wrote the paper. All authors have read, contributed to and approved the final version of the paper.

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SUPPORTING INFORMATION

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

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