# Childhood CCL18, CXCL10 and CXCL11 levels differentially relate to and predict allergy development

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**Abstract**

Background: Chemokines are important mediators in immune cell recruitment, contributing to allergy development. However, extensive studies of chemokines in the circulation in relation to the presence and development of allergic diseases remain scarce. Our aim was to investigate associations of circulating allergy-related chemokines with development of asthma and sensitisation cross-sectionally and longitudinally in a population-based cohort. Methods: The chemokines CCL17, CCL22, CXCL10, CXCL11 and CCL18 were measured in plasma samples from children in the Manchester Asthma and Allergy Study. Samples were available from cord blood at birth (n=376), age 1 (n=195) and 8 years (n=334). Cross-sectional and longitudinal association analyses were performed in relation to asthma and allergic sensitisation, as well as allergic phenotype clusters previously derived using machine learning in the same study population. Results: In children with asthma and/or allergic sensitisation, CCL18 levels were consistently elevated at ages 1 and/or 8 years. In a longitudinal model including information on asthma from 4 time-points (ages 5, 8, 11 and 16 years), we observed a significant association between increasing CCL18 levels at age 1 and a higher risk of asthma from early school age to adolescence (OR=2.9, 95% CI 1.1-7.6, p=0.028). We observed similar associations in longitudinal models for allergic sensitisation. Asthma later in life was preceded by increased CXCL10 levels after birth, and decreased CXCL11 levels at birth. Conclusion: Elevated CCL18 levels throughout childhood precede the development of asthma and allergic sensitisation. The Th1-associated chemokines CXCL10 and CXCL11 also associated with development of both outcomes, with differential temporal effects.

## Childhood CCL18, CXCL10 and CXCL11 levels differentially relate to and predict allergy development

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**Running title: Allergy-related chemokines in childhood**

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## ABSTRACT

**Background:** Chemokines are important mediators in immune cell recruitment, contributing to allergy development. However, extensive studies of chemokines in the circulation in relation to the presence and development of allergic diseases remain scarce. Our aim was to investigate associations of circulating allergy-related chemokines with development of asthma and sensitisation cross-sectionally and longitudinally in a population-based cohort.

**Methods:** The chemokines CCL17, CCL22, CXCL10, CXCL11 and CCL18 were measured in plasma samples from children in the Manchester Asthma and Allergy Study. Samples were available from cord blood at birth (n=376), age 1 (n=195) and 8 years (n=334). Cross-sectional and longitudinal association analyses were performed in relation to asthma and allergic sensitisation, as well as allergic phenotype clusters previously derived using machine learning in the same study population.

**Results:** In children with asthma and/or allergic sensitisation, CCL18 levels were consistently elevated at ages 1 and/or 8 years. In a longitudinal model including information on asthma from 4 time-points (ages 5, 8, 11 and 16 years), we observed a significant association between increasing CCL18 levels at age 1 and a higher risk of asthma from early school age to adolescence (OR=2.9, 95% CI 1.1-7.6, p=0.028). We observed similar associations in longitudinal models for allergic sensitisation. Asthma later in life was preceded by increased CXCL10 levels after birth, and decreased CXCL11 levels at birth.

**Conclusion:** Elevated CCL18 levels throughout childhood precede the development of asthma and allergic sensitisation. The Th1-associated chemokines CXCL10 and CXCL11 also associated with development of both outcomes, with differential temporal effects.

**Keywords:** allergy, asthma, CCL18, chemokine, CXCL10, CXCL11, sensitisation

**Key messages:** Chemokines are highly involved in the development of allergic disease, by promoting recruitment to the allergic reaction site. While previous studies have shown associations of childhood chemokine levels with development of allergic manifestations, we were in this study able to show longitudinal impact of chemokine levels in infancy and childhood on the development of sensitisation and asthma both contemporaneously as well as later in life. This suggests chemokines as important biomarkers for allergic disease, which could potentially be useful in a clinical setting for predicting development of sensitisation and asthma.

## INTRODUCTION

Worldwide, allergic diseases affect development and well-being of many children and adolescents, while also posing a considerable socioeconomic burden.[1] Allergic diseases are heterogenous and may present with similar symptoms but different underlying causes, the features of which are just beginning to be characterised.[2] Both sensitisation and asthma are considered heterogeneous traits, with distinct clusters being unveiled using unsupervised machine learning techniques.[3,4]While numerous immune biomarkers have been investigated as potential determinants of heterogeneity and development of allergic phenotypes, thus far none has proven unequivocal utility in a clinical setting.

Allergic inflammation depends on chemokine-facilitated recruitment of immune cells to the allergic reaction site.[5] The Th2-associated chemokines CCL17 and CCL22 are expressed by the thymus, and may be induced by IL-4 and IL-13 in several cell types including T cells.[6,7] Development of allergic symptoms and sensitisation early in life is preceded by elevated cord blood levels of CCL17 and CCL22, respectively.[8,9] Similar findings from sensitised children with allergic symptoms[8,9], and children developing recurrent wheeze[10] and asthma[9], further corroborate involvement in allergic immune responses.

The Th1-associated chemokines CXCL10 and CXCL11 are induced by IFN-$\gamma$[11], and are mainly expressed by the thymus, peripheral blood leukocytes, epithelial and endothelial cells.[12,13] Increased levels of CXCL10 and/or CXCL11 have been observed in both viral-induced[14] and moderate-to-severe asthma.[15] Multiple studies have also revealed elevated early life Th1-associated chemokine levels predicting outcomes such as wheezing and asthma.[10,16]In contrast, sensitised children revealed lower circulating levels of CXCL11 at birth and 2 years of age[10], suggesting diverse mechanisms of action in allergy development.

CCL18 is under dual regulation of both Th2 and Treg cells, as it may be induced by IL-4, IL-13 as well as IL-10.[17] This contrasts to CCL17 and CCL22, which are inhibited by IL-10.[17,18] Being produced primarily by tissue resident antigen presenting cells, CCL18 is also constitutively expressed in the lung and circulation.[17] At steady state, CCL18 is mainly a regulatory chemokine, which is up-regulated in allergic conditions such as allergic rhinitis, atopic dermatitis and asthma.[17] Indeed, we have previously showed elevated levels of CCL18 in children developing eczema and recurrent wheeze in the first years of life.[10]

We hypothesised that allergy-related chemokines, namely CXCL10, CXCL11, CCL17, CCL22 and CCL18, precede the development of different allergic phenotypes throughout childhood. To this end, we measured circulating levels of these chemokines at three time points throughout childhood (at birth, 1 year and 8 years of age) in a population-based birth cohort[19], and related these chemokines to allergic outcomes from infancy to age 16 years. Furthermore, we ascertained the relationship between these chemokines and previously described clusters of allergic diseases derived using machine learning in this cohort.[20-23]

## METHODS

Details on the materials, methods, definitions of variables and statistical approach may be found in the Supplementary Appendix. Briefly, 905 plasma samples originating from the time of birth (cord blood, n=376), 1 year (n=195) and 8 years (n=334) from the Manchester Asthma and Allergy Study (MAAS) were analysed for their chemokine content (Figure S1). Plasma levels of CCL18 were measured using an in-house DuoSet ELISA kit (R&D Systems) and an in-house multiplex bead assay was setup for analysis of circulating CCL17, CCL22, CXCL10 and CXCL11.

The chemokine levels were thereafter associated to asthma and allergic sensitisation, and CCL18 levels were additionally related to multivariable outcomes. To study whether chemokine levels predicted asthma or aller-

gic sensitisation, binomial logistic regression analyses were performed on natural log transformed chemokine data. Longitudinal analyses were performed using generalised estimating equations (GEE). Population-averaged GEE models were developed to investigate whether the effect of natural log-transformed chemokine levels on the development of asthma or sensitisation changed over time. All models were adjusted for parental atopy, parental smoking and gender. Resulting coefficients represent the increased/decreased odds of the respective outcome per log-unit increase in chemokine levels.

Analyses were conducted in GraphPad Prism 8[24], IBM SPSS Statistics version 25[25], and Stata 15 software.[26]

## RESULTS

### Kinetics of circulating chemokines

The plasma measurements revealed distinctive expression patterns for the five chemokines (Figure 1). The Th2-associated chemokine CCL17 displayed significantly lower levels at 1 and 8 years of age, compared to at birth (Figure 1A). In contrast, the Th2-associated chemokine CCL22 revealed higher concentrations at age 8 compared to earlier in life (Figure 1B). For the Th2/Treg-associated chemokine CCL18 and the Th1-associated chemokine CXCL10, significantly higher levels were evident at 1 and 8 years of age compared to at birth (Figure 1C-D). However, the expression was significantly lower at age 8 than at age 1 for both chemokines. The Th1-associated chemokine CXCL11 showed significantly higher levels at age 8 compared to the earlier time points (Figure 1E).

### CXCL10, CXCL11 and CCL18 levels associate with asthma and sensitisation

As the investigated chemokines have previously been related to allergy development, we performed cross-sectional analyses in relation to outcomes of asthma (Figures 2 and S2) and sensitisation (Figures 3 and S3) in childhood and adolescence.

*Asthma:* CXCL10 levels were significantly increased at age 1 year in children with asthma at the same age (Figure 2A). Furthermore, children with asthma at ages 8 and 11 years exhibited elevated CXCL10 levels at age 8 (Figure 2B, Figure S2A). In contrast, CXCL11 was significantly decreased in cord blood of children having asthma at age 16 (Figure S2B). For CCL18, higher levels at age 8 associated with asthma at ages 8 and 16 years (Figure 2C-D).

*Sensitisation:* CXCL10 levels in cord blood were significantly lower than in non-sensitised children at ages 1 and 16 years (Figure 3A-B). Similarly, CXCL11 levels at 1 year and at birth were significantly elevated in non-sensitised children at 1 and 16 years of age, respectively (Figure S3A-B). Children who were sensitised at ages 1, 8, 11 and 16 years consistently displayed significantly elevated concentrations of CCL18 at 1 and/or 8 years compared to non-sensitised children (Figure 3C-E, Figure S3C). Increased levels of the Th2-associated chemokine CCL17 at birth associated with sensitisation at age 8 (Figure S3D).

### CXCL10, CXCL11 and CCL18 predict asthma and sensitisation

As some of the chemokines indeed associated with the development of asthma and sensitisation later in childhood, we tested the ability of the chemokine levels to predict asthma (Figure 4A) and sensitisation (Figure 4B) later in childhood and adolescence using binomial logistic regression models.

*Asthma:* Having higher CXCL11 levels at birth constituted lower odds of being asthmatic at age 16 (OR=0.4, 95% CI 0.2-0.8, p=0.012). Furthermore, higher CCL18 levels at 8 years of age markedly increased the odds of being asthmatic at ages 8 (OR=3.8, 95% CI 1.5-9.8, p=0.006) and 16 years (OR=5.3, 95% CI 1.7-16.1, p=0.003).

*Sensitisation:* Presenting with elevated CCL18 levels at age 8, posed a three times or higher odds ratio for developing sensitisation at ages 8 (OR=3.3, 95% CI 1.5-7.1, p=0.002), 11 (OR=3.0, 95% CI 1.3-6.7, p=0.008) and 16 years of age, (OR=4.2, 95% CI 1.7-10.5, p=0.002). In contrast, having high levels of CXCL10 at age 8 seemed protective against becoming sensitised at age 11 (OR=0.4, 95% CI 0.2-0.8, p=0.012).

### Association between CCL18 levels and the development of asthma and sensitisation

Proceeding with the chemokines showing the strongest consistent associations cross-sectionally, we set up GEE models studying each chemokine at each age separately in relation to various intervals of asthma and sensitisation development to study longitudinal relationships over time. These models take into account correlations within individuals, and as the data are not independent over time, this provides an advantage of running both the GEE and logistic regression models. Consistent longitudinal patterns were only revealed for circulating levels of CCL18 at ages 1 and 8 (Figure 5).

*Asthma:* In a longitudinal model including information on asthma from four time-points (ages 5, 8, 11 and 16), we observed a significant association between increasing CCL18 levels at age 1 year and the higher risk of asthma from early school age to adolescence (OR=2.9, 95% CI 1.1-7.6, p=0.028). Similarly, higher CCL18 levels at ages 1 (OR=3.5, 95% CI 1.3-9.8, p=0.01) and 8 years (OR=3.0, 95% CI 1.6-5.9, p=0.001) were associated with an increased risk of asthma between ages 8 and 16 years.

*Sensitisation:* In a longitudinal model including information on SPTs from five time-points (ages 3-16 years), the odds of becoming sensitised increased significantly with increased CCL18 concentrations at age 1 (OR=3.1, 95% CI 1.2-8.3, p=0.022). Similarly, the odds ratios for developing sensitisation between the ages 8 and 16 years increased significantly with increasing CCL18 levels at ages 1 (OR=3.5, 95% CI 1.3-9.9, p=0.018) and 8 years (OR=3.0, 95% CI 1.5-5.9, p=0.002).

**Association of CCL18 levels with clusters of allergic diseases**

As we previously have derived clusters of allergic sensitisation[20,21], allergic diseases[22] and asthma exacerbations[23] from children in the MAAS cohort using machine learning, we sought to study the relationship between the chemokine levels and these putative endotypes of allergic diseases.

Children belonging to the multiple early sensitisation cluster[20] had significantly higher levels of CCL18 at age 8 compared to non-atopic subjects (Figure 6A). In relation to CRD sensitisation patterns[21], circulating levels of CCL18 at age 8 were elevated in individuals sensitised towards multiple allergens as compared to children with predominant grass and tree sensitisation, and as a trend compared to children who were sensitised to a lesser degree (Figure 6B).

There was a trend towards higher levels of CCL18 at age 8 both in the eczema only and the atopic march clusters compared to the cluster consisting of healthy children[22] (Figure 6C).

Associating chemokine levels to developmental pattern of asthma exacerbations in the first 8 years of life, children presenting with exacerbations[23] showed significantly higher levels of CCL18 at age 8 compared to non-wheezers (Figure 6D).

None of the other chemokines revealed any differences between the investigated clusters.

## DISCUSSION

In this study, we show that levels of the chemokines CCL18, CXCL10 and CXCL11 in early life and childhood may predict outcomes of allergic disease later in childhood and adolescence. To our knowledge, this is the first study to show that childhood circulating CCL18 levels may affect allergy-related outcomes longitudinally until adolescence.

The main finding was that the dually Th2/Treg regulated chemokine CCL18 predicted development of both asthma and sensitisation, with consistent effects over time. Being constitutively expressed in the lung and lymphoid tissues during homeostatic conditions, CCL18 exhibits both chemotactic and immunoregulatory properties.[17] It promotes tolerogenic differentiation of dendritic cells, which in turn may polarise T cells into Tregs, and may polarise memory T cells into FoxP3+ T cells *in vitro* . In allergic subjects, however, the tolerogenic effects of CCL18 are seemingly abrogated, despite being upregulated in allergic conditions such as atopic dermatitis[10,27,28] and asthma.[17]Described being due to less efficient binding of the protein on immune cells, this possibly could partly explain the loss of tolerance in allergic individuals. Furthermore, CCL18 induces production of collagen both in the skin and lung, implying a role in remodelling of the airways typically seen in asthmatic subjects.[17] As alveolar macrophages are the main producers of CCL18 in

the lung, where its expression is constitutive, it is tempting to speculate that these levels are augmented in asthmatic individuals owing to dysregulation of these cells. However, as our measurements were performed in plasma samples, and CCL18 may originate from one of many bodily sources, we cannot draw conclusions on tissue specific effects of the observed elevation without performing functional studies. Furthermore, whether heightened CCL18 responses in asthmatic and sensitised children constitute causative mechanisms of allergy induction, or compensatory immune dampening responses, remains to be elucidated. We further examined chemokine expression within allergy clusters previously derived from our cohort.[20-23] Indeed, CCL18 levels at age 8 were higher in the multiple early allergic sensitisation cluster. Moreover, children with asthma exacerbations had higher levels of CCL18 at age 8 compared to children without wheeze. Taken together, this suggests that increased CCL18 levels later in childhood may reflect allergic disease severity, although further studies should elaborate on this matter.

Interesting findings also appeared for the Th1-associated chemokines CXCL10 and CXCL11. Elevated levels of CXCL10 in infancy and childhood associated with present and future development of asthma, in line with results from children with wheezing at age 3, who subsequently developed asthma at age 6.[16] Additionally, CXCL10 levels are increased in viral-induced asthma[29,30], suggesting that viral infections may induce Th1-chemokine responses in asthmatic individuals. On the contrary, low cord blood CXCL10 levels associated with sensitisation in infancy and adolescence. Similarly, decreased CXCL11 levels in early life associated with later development of sensitisation. This corroborates findings from our previous studies, where SPT-positive children had lower levels of CXCL11 at birth and 24 months.[10] As sensitisation is a Th2-driven process, and Th1-responses were lessened in sensitised children, diminished neonatal Th1-responses seemingly paves way for development of sensitisation in these children. Additionally, reduced cord blood CXCL11 levels associated with asthma at age 16 and translated into a predicted lower risk with high CXCL11 levels at birth, supporting previous results where children with the highest quartile CXCL11 levels at birth did not become sensitised throughout the first two years of life.[10] No long-term effects of CXCL10 and CXCL11 on allergy development could be demonstrated in this study. Possibly, the function of Th1 cells, and their expression of IFN-$\gamma$, may become attenuated due to immunoregulatory effects of the highly expressed CCL18 on Tregs, although the findings may also constitute altered patterns of expression in allergic conditions. Collectively, this indicates that although these chemokines are induced by the same cytokine, downstream effects seem to be differentially regulated both in terms of allergy outcome and how levels reflect temporal development of disease.

There are both limitations and strengths to the present study. We evaluated circulating chemokine levels but did not have the opportunity to evaluate functional aspects of the same mediators in different tissues. This would have added mechanistic insights into the findings presented here. Also, the generalisability of these data may be limited, as children in the cohort originate from the Greater Manchester region, with rather homogenous populations. A strength of this study includes the substantial sample size, as few studies have surveyed circulating chemokines at this magnitude. Furthermore, the consistency of the methodologies used compared to previous studies provides another advantage. Moreover, by performing both cross-sectional logistic regression and longitudinal GEE models we have taken into account different temporal perspectives throughout childhood, which is a strength of this study.

In conclusion, we have shown that elevated levels of CCL18 throughout childhood precede the development of asthma and sensitisation, findings that remained solid longitudinally. The Th1-associated chemokines CXCL10 and CXCL11 also predicted development of sensitisation and asthma, with differential regulation at different time points in life. This motivates further investigations of chemokines as biomarkers for allergy development, with putative clinical utility in the prediction of allergic outcomes.

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**Impact statement**

The findings presented in this study could, upon careful validation, provide the basis of chemokines as biomarkers for predicting the development of allergic manifestations throughout childhood. These may furthermore provide clues to the underlying causes of childhood allergies, all in all promoting the understanding of how to predict, prevent and treat allergic diseases.

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## FIGURE LEGENDS

**Figure 1.** Circulating levels of chemokines from children in the MAAS cohort. Plasma concentrations of A. CCL17, B. CCL22, C. CCL18, D. CXCL10 and E. CXCL11 were measured by means of Luminex and ELISA methodology at birth (in cord blood), age 1 and age 8. The data are displayed as medians with interquartile ranges. Statistical differences were ascertained using a Kruskal-Wallis test with a Dunn's post hoc test for multiple comparisons. *** $p < 0.001$. CB – cord blood.

**Figure 2.** Associations of the chemokines CXCL10 and CCL18 to asthma development. Plasma concentrations of the Th1-associated chemokine CXCL10 are displayed in relation to development of asthma at age 1 in A and age 8 in B. Circulating levels of the Th2/Treg-associated chemokine CCL18 in relation to

asthma development at age 8 and 16 years are illustrated in C and D, respectively. Asthma was defined as fulfilling at least two out of three criteria at the investigated time point: current wheeze, current use of asthma medication, or physician-diagnosed asthma. The data are presented as medians with interquartile ranges. Mann-Whitney $U$ tests were performed to survey statistical significance. * $p < 0.05$, *** $p < 0.001$. CB – cord blood.

**Figure 3.** Associations of the chemokines CXCL10 and CCL18 to allergic sensitisation. Plasma concentrations of the Th1-associated chemokine CXCL10 are displayed in relation to development of sensitisation, at age 1 in A and age 16 years in B. Circulating levels of the Th2/Treg-associated chemokine CCL18 in relation to sensitisation at age 1, 8 and 16 years are illustrated in C, D and E, respectively. Sensitisation status was determined by means of skin prick testing. The data are displayed as medians with interquartile ranges. Mann-Whitney $U$ tests were performed. * $p < 0.05$, ** $p < 0.01$. CB – cord blood.

**Figure 4.** Forest plots of odds ratios from logistic regression models on predicting allergy development from circulating chemokine levels. Probabilities of developing asthma as depicted in A and sensitisation as illustrated in B from cross-sectional adjusted logistic regression models including all chemokines and correction for the confounding factors sex, parental atopy and parental smoking. Odds ratios are denoted with crosses and the corresponding values below them, and 95% confidence intervals are illustrated by the error bars. All displayed models have an adjusted p-value of <0.05.

**Figure 5.** Forest plot of odds ratios from generalised estimation equation (GEE) models predicting longitudinal allergy development from the measured chemokines. In the GEE-models, the predictive ability of the chemokine CCL18 on longitudinal development of asthma at ages 5-16 years and 8-16 years, as well as sensitisation at 3-16 years and 8-16 years of age, was examined. Odds ratios are denoted with crosses and the corresponding values below them, and 95% confidence intervals are illustrated by the error bars. All displayed models revealed an adjusted p-value of <0.05.

**Figure 6.** Associations of CCL18 levels at age 8 to previously machine learning derived clusters of allergy outcomes from the MAAS cohort. Panel A displays sensitisation clusters, B CRD IgE clusters, C atopic diseases clusters and D exacerbations clusters. The data are presented as medians with interquartile ranges. A Kruskal-Wallis test with Dunn's post hoc test for multiple comparisons was performed. * $p < 0.05$, ** $p < 0.01$. CRD – component resolved diagnostics.