KØBENHAVNS UNIVERSITET

Asthma and allergy during first 18 years of life - better understanding of endotypes and better prevention of disease

PhD Thesis

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Abbreviations

AR – Allergic rhinitis

AUC – Area under the receiver operating characteristic curve

BHR – Bronchial hyperresponsiveness

BH – Benjamini–Hochberg (procedure)

CI – Confidence interval

COPSAC – Copenhagen Prospective Studies on Asthma in Childhood

CRD – Component-resolved diagnostics

DNN – Deep neural network(s)

ENet – Elastic Net (regularized regression)

e1 – Cat whole-extract specific IgE (ImmunoCAP code e1)

e5 – Dog whole-extract specific IgE (ImmunoCAP code e5)

ERS – European Respiratory Society

FDR – False discovery rate

FEF_{50} – Forced expiratory flow at 50% of expired volume

FeNO – Fractional exhaled nitric oxide

$FEV_{0.5}$ – Forced expiratory volume in 0.5 seconds

FEV_1 – Forced expiratory volume in 1 second

FVC – Forced vital capacity

HDM – House dust mite

ICS – Inhaled corticosteroids

IgE – Immunoglobulin E

IL-6 – Interleukin-6

ISAC – Immuno Solid-phase Allergen Chip

ISU – ISAC Standardized Units

LightGBM (LGBM) – Light Gradient Boosting Machine

LMM – Linear mixed model(s)

$\log_2$ – Base-2 logarithm

MMEF – Maximal mid-expiratory flow

NO_2 – Nitrogen dioxide

OR – Odds ratio

PD_{15} – Provocative dose causing a 15% decrease in transcutaneous oxygen pressure

PD_{20} – Provocative dose causing a 20% fall in FEV_1

ppb – Parts per billion

RF – Random forest

SD – Standard deviation

sIgE – Specific immunoglobulin E

SHAP – SHapley Additive exPlanations

SPT – Skin prick test(ing)

sRaw – Specific airway resistance

T2 – Type 2 (inflammation)

UpSet – UpSet plot (set-intersection visualization)

Wald test – Wald test

Z-score – Standardized score

Allergen component nomenclature used in the thesis

Can f 1–6 – Dog allergen components (Canis familiaris)

Equ c 1 / Equ c 3 – Horse allergen components (Equus caballus)

Fel d 1 / Fel d 2 / Fel d 4 – Cat allergen components (Felis domesticus)

d1 – House dust mite allergen component (Dermatophagoides pteronyssinus)

Units and symbols

kPa·s – Kilopascal-seconds

kU/L – Kilounits per liter

kUA/L – Kilounits of allergen-specific IgE per liter

β – Regression coefficient (beta)

β_2-agonist – Beta-2 agonist (bronchodilator)

Introduction

Research environment

During my PhD, I worked clinically across both $COPSAC_{2000}$ and $COPSAC_{2010}$, including responsibility for the 13-year visit in $COPSAC_{2010}$ and in the ABC satellite clinic in Næstved. I contributed to recruitment and assessments in $COPSAC_{Severe}$, the vitamin D trial, and the azithromycin study. Furthermore, I undertook extensive data harmonization, calibration of lung function outcomes, and objective validation of asthma diagnoses. I was also actively involved in securing funding for COPSAC activities and obtained support for a four-month research stay in BC Children's Hospital in Vancouver, Canada, where I contributed to data collection for the DINOSAUR study.

Background

Asthma and allergic rhinitis (AR) are common chronic inflammatory conditions in childhood and adolescence and are associated with substantial symptom burden, impaired quality of life, and long-term respiratory morbidity (1,2). Both conditions may present early in life, fluctuate over time, and persist into adulthood (3,4). However, their long-term trajectories are highly heterogeneous: while some children experience transient symptoms that resolve during childhood, others develop persistent disease with ongoing morbidity and long-term respiratory consequences (3,5). Understanding why disease courses diverge so markedly between children remains a central challenge in pediatric research.

Increasing attention has therefore turned to underlying biological mechanisms that may help explain differences in disease expression and long-term prognosis. In asthma, type 2 (T2) inflammation - characterized by eosinophilic inflammation, elevated fractional exhaled nitric oxide (FeNO), and IgE-mediated immune responses (6–11) - has been associated with greater disease severity (8,10,11), increased airway hyperresponsiveness (5,12), impaired lung function (5,10,13), and a reduced likelihood of remission (8). In children, T2 inflammation is linked to more airway hyperresponsiveness and a higher risk of exacerbations (14). However, the clinical

implications of T2 inflammation in childhood, particularly in relation to long-term lung function development and disease persistence, remain incompletely understood (15,16).

As allergic sensitization forms part of the T2 inflammatory profile, it may contribute to the association between T2 inflammation and airway dysfunction. Sensitization to aeroallergens typically increases across childhood, with prevalence often rising into adolescence. Among these, sensitization to furry animals is of particular clinical interest because exposure to pet allergens is widespread, persistent, and not restricted to pet-owning household (17,18). Studies have linked sensitization and exposure to furry pets to increased bronchial hyperresponsiveness (BHR) and greater airway inflammation (19–23), and increased risk of asthma (24). However, only a few studies have examined the impact of pet sensitization on objective lung function measures in adolescence.

In clinical practice, sensitization is typically assessed using skin prick testing or specific IgE to whole allergen extracts (25). Because extracts contain multiple allergenic proteins, these tests reflect an aggregate IgE response and cannot distinguish primary sensitization from cross-reactive IgE to homologous proteins shared across species (26,27,22). Consequently, individuals with similar whole-extract sensitization profiles may differ substantially in their underlying molecular sensitization patterns (27,28).

Component-resolved diagnostics (CRD), including the ImmunoCAP ISAC platform (ISAC) (29), enable assessment of IgE to individual allergen molecules and have demonstrated marked heterogeneity within pet allergy - particularly for dog, where sensitization patterns are more diverse than for cat (27,28,30). Component-based sensitization to cat has been associated with stronger airway responsiveness and a higher risk of subsequent asthma or AR compared with whole-extract sensitization (19). A key unanswered question is whether molecular profiling provides incremental information beyond whole-extract sensitization in relation to objective airway outcomes in adolescence, including lung function, airway inflammation and BHR, and whether any potential added value is detectable in population-based cohort settings.

In addition to biological characterization, an important clinical goal is early identification of children at risk of persistent asthma or AR. Over the past decades, numerous early-life factors

have been linked to later disease (31,32), including familial or genetic susceptibility (33), environmental exposures (5,34), allergic sensitization (5,35–38), and early symptoms (37,38). These associations have provided important insight into pathways of disease development and progression. However, although such factors are consistently associated with increased risk at the population level, they offer limited precision when applied to the individual child. Children with seemingly similar early-life profiles may go on to experience markedly different disease courses.

Part of this challenge is methodological. Even when several early-life factors are included in the same analysis, models typically require researchers to define in advance which predictors and interactions to test. This approach works well when hypotheses are clearly specified, but in complex longitudinal settings - where biological, environmental, and clinical factors evolve and overlap across childhood - it becomes difficult to foresee all potentially relevant combinations. This has motivated the use of analytical approaches such as machine learning, which are capable of evaluating many measures jointly and linking early-life data to outcomes observed years later. Rather than focusing on a limited set of predefined relationships, these methods enable broader exploration of the data, allowing combinations of variables to be assessed simultaneously and recurrent patterns to emerge empirically (39).

In childhood asthma and AR, genetic susceptibility, environmental exposures, immune development, and early symptoms interact across development, and their relative importance may shift over time. This dynamic interplay further complicates early prediction. Machine learning therefore offers an opportunity to explore how these factors combine and whether specific early-life patterns are more common among children whose disease persists. At the same time, applying such methods in a well-characterized birth cohort makes it possible to evaluate the realistic limits of prediction in a complex, multifactorial disease where no single factor is sufficient to determine long-term outcome.

Aim of the thesis

The overall aim of this thesis was to investigate the development, heterogeneity, and long-term outcomes of asthma and AR from childhood to early adulthood using data from the $COPSAC_{2000}$ birth cohort. Specifically, the thesis aimed to:

- characterize T2-high and T2-low asthma phenotypes and their clinical and physiological trajectories across childhood (**Study I**),
- evaluate the potential of early-life data to predict persistent asthma and AR using machine learning approaches (**Study II**), and
- examine molecular patterns of allergic sensitization and their associations with lung function and airway inflammation in adolescence (**Study III**).

Method

All three studies in this thesis are based on the Copenhagen Prospective Study on Asthma in Childhood 2000 ($COPSAC_{2000}$), which is a single-center, prospective birth cohort of 411 children born to mothers with a history of asthma between 1998 and 2001. Infants were enrolled at one month of age and followed at the COPSAC clinical research unit with scheduled visits every six months from birth to seven years, and follow-up visits at 13 and 18 years. In addition, children were seen at the clinic during episodes of acute troublesome lung symptoms or skin flare ups. The cohort design, visit schedule, and clinical algorithms are described in detail in the original cohort (40).

Ethics

The $COPSAC_{2000}$ study was approved by the Copenhagen Ethics Committee (KF 01-289/96) and the Danish Data Protection Agency (2015-41-3696). Oral and written informed consent was obtained from both parents at enrollment. At the 18-year visit, participants provided their own written consent. All procedures followed the Declaration of Helsinki and Good Clinical Practice guidelines.

Study populations

For this thesis, three overlapping study populations were defined:

- **Study I** included children with a confirmed asthma diagnosis at age 7 years and with available biomarker measurements at the 7-year visit (aeroallergen sensitization, FeNO, and blood eosinophils). The control group comprised children with no history of asthma who attended at least one visit between ages 0 and 6.5 years and the protocol visits at 7, 13, and 18 years.

- **Study II** included all children from the $COPSAC_{2000}$ cohort with available early-life data collected between birth and 7 years of age and with asthma and AR status assessed at the 18-year follow-up. Only children with missing outcome data at 18 years or insufficient early-life information to allow inclusion in the machine learning analyses were excluded.

- **Study III** included participants who attended the 18-year follow-up visit and had available ISAC molecular allergen measurements. Children were excluded only if the ISAC results from the 18-year visit were missing.

Data collection and objective measurements

Blood eosinophils and total IgE

Blood samples were obtained at scheduled visits throughout childhood and adolescence. Absolute blood eosinophil counts were derived from routine blood cell counts, and total serum IgE was quantified using ImmunoCAP (Thermo Fisher Scientific), as previously described in the COPSAC cohort (40,41).

Sensitization

Sensitization to inhalant allergens was assessed repeatedly throughout childhood by skin prick testing (SPT; ALK-Abelló) and serum specific IgE (sIgE; ImmunoCAP, Thermo Fisher Scientific) (40,42).

- A positive SPT was defined as a wheal diameter ≥3 mm above the negative control, and
- sIgE levels ≥0.35 kU/L were considered indicative of sensitization.

These measures were applied across all three studies for case definitions and exposure variables and were used as comparators in analyses of molecular sensitization.

Component-resolved diagnostics

CRD were performed using the Immuno Solid-phase Allergen Chip (ISAC), a microarray-based platform assessing IgE sensitization to individual allergen molecules (29,43). ISAC measurements were available across several follow-up visits; however, analyses in this thesis focused on data from ages 7, 13, and 18 years, as sensitization to pet allergen components was uncommon earlier in childhood.

ISAC values were reported in ISAC Standardized Units (ISU), and sensitization was defined as ISU ≥0.30 in accordance with established recommendations (29).

For Study III, analyses focused on molecular sensitization to pet allergen components:

- Dog: Can f 1, Can f 2, Can f 3, Can f 4, Can f 5, Can f 6

- Cat: Fel d 1, Fel d 2, Fel d 4
- Horse: Equ c 1, Equ c 3

Parallel sIgE (cat e1, dog e5) and SPTs (cat, dog, horse) were used to compare molecular and conventional sensitization measures.

Lung function, airway responsiveness, and inflammation

Lung function, airway responsiveness, and airway inflammation were assessed repeatedly throughout childhood and adolescence.

At 1 month of age, neonatal lung function was assessed by infant spirometry using the raised-volume rapid thoracoabdominal compression technique, yielding forced expiratory volume in 0.5 seconds ($FEV_{0.5}$) and forced expiratory flow at 50% of expired volume (FEF_{50}) (44). Bronchial responsiveness (BHR) was evaluated during the same procedure by methacholine challenge and expressed as PD_{15}, defined as the provocative dose causing a 15% decrease in transcutaneous oxygen pressure (45,46).

From age 3 years, specific airway resistance (sRaw) was measured by whole-body plethysmography at scheduled follow-up visits and during acute clinic visits for respiratory symptoms (47,48). From age 5 years, spirometry was introduced, including forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), and maximal mid-expiratory flow (MMEF) (40,48).

BHR beyond infancy was assessed at selected ages using age-appropriate challenge modalities. Methacholine challenge testing was performed at ages 6.5, 13, and 18 years and expressed as PD_{20}, defined as the provocative dose causing a 20% fall in FEV_1 (49).

At age 6 years, BHR was evaluated using a cold dry air challenge, defined as a ≥10% fall in FEV_1 (50). At age 7 years, BHR was assessed using a standardized exercise challenge, defined as a ≥10% fall in FEV_1 following exercise (51).

Bronchodilator reversibility testing was performed after inhalation of a short-acting β_2-agonist (Bricanyl 0.25 mg per puff or Ventoline 0.1 mg per puff; AstraZeneca) at visits where no

bronchial challenge test was conducted, as well as during unscheduled acute visits when children presented with asthma symptoms or suspected loss of disease control. Reversibility was defined as a ≥12% improvement in FEV_1 (52) and/or a ≥30% reduction in sRaw (53).

Airway inflammation was assessed by measurement of fractional exhaled nitric oxide (FeNO) at ages 5, 6, 7, 13, and 18 years using NIOX Flex (Aerocrine, Solna, Sweden) (41).

An overview of the timing and type of lung function and airway responsiveness measurements obtained across childhood and adolescence in the $COPSAC_{2000}$ cohort is presented in Figure 1.

Figure 1

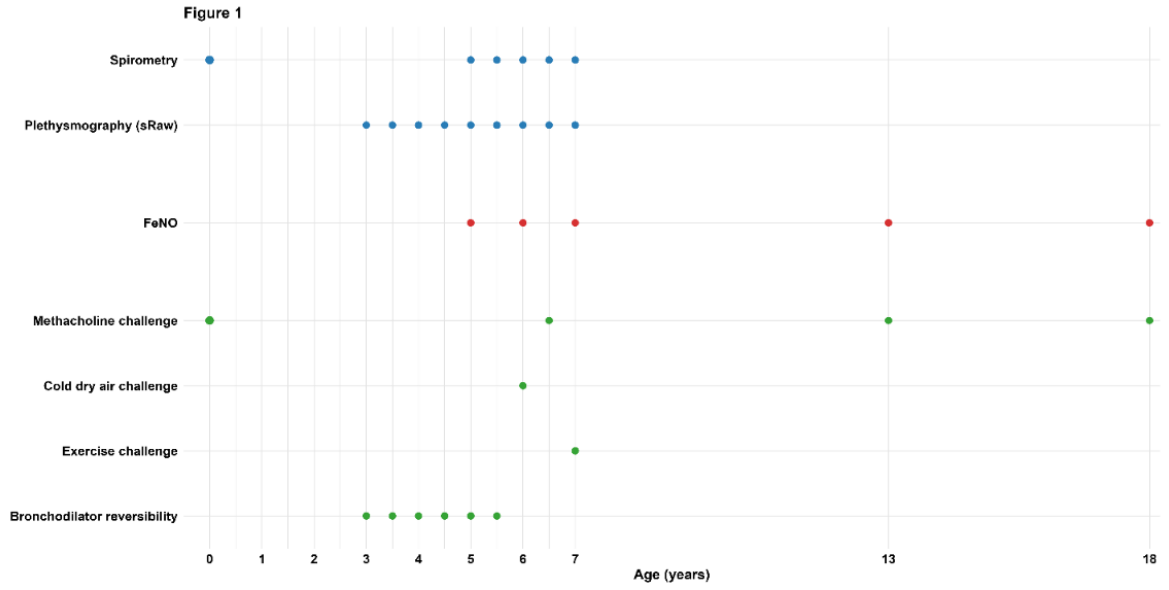


Figure 1. Overview of the timing and type of lung function, airway responsiveness, and airway inflammation measurements across childhood and adolescence in the COPSAC2000 cohort. Colors indicate different measurement modalities, including lung function and airway resistance (blue), airway inflammation (red), and bronchial challenge and reversibility testing (green).

To enable consistent comparison of lung function, airway responsiveness and inflammatory markers across childhood and adolescence, all physiological outcomes were systematically processed and calibrated prior to analysis, as previously applied in the $COPSAC_{2000}$ cohort (48). FEV_1, FEV_1/FVC-index, FVC, MMEF and sRaw were calibrated for age, sex, height and the presence of a current respiratory infection (e.g., symptoms of airway infection within the preceding seven days), after which residuals were centered to obtain standardized outcomes.

Measures with skewed distributions, including sRaw, PD_{20} and FeNO, were log-transformed before modelling. For Study I, Z-scores for FEV_1, MMEF, sRaw, and BHR (PD_{15}/PD_{20}) were generated using children without asthma as the reference population. These procedures ensured comparability of measurements obtained across different ages and timepoints over two decades and supported longitudinal analyses.

Clinical definitions

Asthma

Asthma was diagnosed and managed solely by COPSAC physicians according to a predefined and prospectively applied clinical algorithm integrating symptom burden and treatment response (40,54).

Clinical suspicion of asthma was based on recurrent troublesome lung symptoms recorded prospectively in daily diaries and verified at scheduled and acute clinic visits. Criteria included:

- recurrent episodes of troublesome lung symptoms, defined as ≥5 episodes lasting ≥3 consecutive days within 6 months, or daily symptoms for ≥4 weeks, and/or
- typical asthma symptoms such as prolonged nocturnal cough, exercise-induced symptoms, or nighttime awakenings due to respiratory symptoms, and/or
- clinical response to intermittent inhaled β_2-agonists, and/or
- relapse of symptoms following a standardized 3-month trial of inhaled corticosteroids (ICS)

Severe exacerbations requiring hospitalization or treatment with oral corticosteroids resulted in the immediate initiation of a 6-month course of ICS and the establishment of an asthma diagnosis.

From age 7 years onwards, all cases were objectively validated using calibrated assessments of lung function, bronchodilator reversibility, airway inflammation and BHR. In accordance with the European Respiratory Society (ERS) clinical practice guidelines for the diagnosis of asthma in children aged 5–16 years (55). Objective confirmation required fulfilment of at least two of the following categories at any clinic visit:

1. Obstructive lung function, defined as FEV_1 <80 percent predicted or FEV_1/FVC <80 percent, and/or elevated sRaw (sRaw ≥1.6 kPa·s)

2. Bronchodilator reversibility, defined as ≥12 percent improvement in FEV_1 or ≥30 percent reduction in sRaw after inhalation of a β_2-agonist
3. Airway inflammation, defined as FeNO ≥25 ppb
4. Bronchial hyperresponsiveness, defined as a ≥20 percent fall in FEV_1 at a methacholine dose ≤8 mg/mL or ≥10 percent decrease in FEV_1 during standardized exercise challenge

Asthma remission was defined as the absence of asthma symptoms and no requirement for asthma medication for one year (48). For this PhD, persistent asthma was defined as asthma with ongoing symptoms and/or maintenance treatment beyond age 17 years in children with a previous diagnosis of childhood asthma. Asthma with remission was defined as children who fulfilled the criteria for childhood asthma but no longer met the diagnostic criteria at or beyond age 17 years. Children with asthma onset after age 17 years and no prior history of childhood asthma were not classified as having persistent asthma.

Allergic rhinitis

AR was assessed at 7, 13, and 18 years. The diagnosis required characteristic nasal or ocular symptoms affecting daily well-being in the absence of a cold together with sensitization to the corresponding aeroallergen (positive SPT and/or sIgE ≥0.35 kU/L) (41). Children with symptoms but no sensitization were classified as non-AR.

Persistent AR was defined as AR present at both 7 and 18 years. Teen-onset AR was defined as no AR at 7 years, followed by AR at 13 and/or 18 years.

T2-high and T2-low asthma

Asthma at age 7 years was classified into T2-high and T2-low phenotypes based on three biomarkers measured at the 7-year visit (9):

- FeNO ≥20 ppb
- Blood eosinophils ≥0.5 × 10^9/L
- Sensitization to aeroallergens (positive SPT and/or sIgE ≥0.35 kU/L)

Asthma was classified as T2-high if at least one of these criteria was fulfilled; T2-low asthma required all three markers to be below the respective thresholds. The eosinophil threshold was set at 0.5×10^9/L to increase specificity for T2 inflammation in this high-risk cohort.

Exacerbations

Exacerbations were registered prospectively and defined as acute severe episodes of wheeze or asthma requiring systemic corticosteroids or a temporary course of high-dose ICS, or hospital admission for obstructive airway symptoms, according to predefined COPSAC procedures (33,54). In children not previously diagnosed with asthma, such episodes triggered a clinical asthma diagnosis and initiation of a 6-month ICS treatment course in accordance with the study algorithm.

Early-life exposures and machine learning feature set

For Study II, prospectively collected early-life data from $COPSAC_{2000}$ were assembled into a unified analytical dataset. Although several variables had been validated in previous studies, the underlying information was distributed across multiple source files and formats. All relevant datasets were therefore merged, harmonized, and cross-checked to ensure consistency across timepoints, coding, and units.

The final feature set comprised 113 variables including clinical history, atopic manifestations, growth and biometric measures, perinatal factors, environmental and exposome data, familial predisposition, immunological markers, lung function and BHR, pregnancy-related exposures, and socioeconomic factors. Given the breadth of the dataset, individual variables are not detailed in the main text; a complete overview of included features and their corresponding measurement timepoints is provided in Supplementary Table E1, Study II.

To avoid predictor–outcome overlap, variables directly incorporated in diagnostic definitions were excluded prior to modelling. Specifically, lung function measures used for objective asthma validation (n = 11) were excluded from the persistent asthma versus control analyses, and sIgE and ISAC measures forming part of the AR definition (n = 14) were excluded from the rhinitis

models. These variables were retained in analyses where they were not involved in defining the outcome.

Statistical analyses

Study I

Analyses in Study I were conducted using classical statistical methods. Categorical outcomes were compared using Fisher's exact test, while continuous variables with non-normal distributions were analyzed with Wilcoxon rank-sum tests. Exacerbation rates were modelled using Quasi-Poisson regression. Longitudinal measurements of lung function and blood eosinophils were analyzed using linear mixed models with random intercepts to account for within-child correlation, and binary repeated outcomes such as bronchodilator reversibility were evaluated using generalized linear mixed models. Age at assessment was included as a fixed effect in longitudinal analyses where appropriate. Model structures were selected based on likelihood ratio tests. For interpretability, estimates from log-transformed outcomes (e.g. sRaw, FeNO, PD_{20}) were back-transformed and presented as percentage differences.

Study II

We employed supervised machine learning to predict persistent asthma and AR trajectories from early-life exposures. Prior to model training, we applied structured pre-feature selection by excluding features with low variance, highly correlated predictors (Spearman's $r > 0.90$), and features with extreme imbalance, resulting in 83 early-life predictors included in the models.

Four supervised machine learning algorithms were implemented: Random Forest (56), Elastic Net logistic regression (57), Light Gradient Boosting Machine (58), and Deep Neural Networks (59).

Data were randomly split into a 60% training set and a 40% independent test set. Hyperparameter optimization was performed using five-fold cross-validation combined with Bayesian optimization (60).

Model performance was evaluated using the area under the receiver operating characteristic curve (AUC). An AUC of 0.5 was interpreted as performance equivalent to chance, and values ≤ 0.6 were considered to reflect insufficient predictive performance at a population level, in line with recommendations on interpretation of discrimination metrics (61).

To enhance model interpretability, permutation-based feature elimination was performed during training. Subsequently, SHapley Additive exPlanations (SHAP) were used to quantify both global and individual predictor contributions (62). SHAP dependence plots were applied to explore non-linear relationships and potential feature interactions.

Study III

Sensitization patterns were explored using UpSet plots to visualize co-sensitization across ISAC pet allergen components. Correlation patterns between molecular components, whole-extract specific IgE, SPT responses, AR symptoms, and HDM sensitization were examined using Spearman correlation coefficients and presented as heatmaps. Differences in sensitization prevalence according to current asthma status were assessed using Fisher's exact test due to small expected cell counts for several allergen components.

Associations between sensitization and objective respiratory outcomes at 18 years were examined using regression models. Continuous outcomes (FEV_1, FEV_1/FVC, MMEF, sRaw, FeNO, and PD_{20}) were analyzed using linear regression, whereas current asthma was analyzed using logistic regression. Due to skewed distributions, sRaw, FeNO, and PD_{20} were analyzed on the $\log_2$ scale, and back-transformed estimates are presented as fold-changes.

ISAC component sensitization was modelled primarily as a binary exposure (≥0.30 ISU). Whole-extract sensitization was analyzed using the same modelling framework. All primary models were adjusted for current smoking and current exposure to the corresponding animal. To account for potential confounding by co-sensitization, models were additionally fitted with and without adjustment for HDM sensitization.

Robustness of the findings was evaluated in several complementary analyses. First, models were repeated using continuous ISAC component levels ($\log_2$(ISAC + 0.1)). Second, analyses were repeated separately among adolescents with and without current asthma to explore potential subgroup-specific effects. Formal interaction between sensitization and asthma status was assessed by including product terms in the regression models, with statistical significance evaluated using Wald tests.

To evaluate whether molecular component sensitization provided additional explanatory value beyond whole-extract sensitization, nested regression models were constructed within each animal species. Base models included whole-extract sensitization, and extended models additionally included the relevant molecular component. Model fit was compared using formal nested model comparisons (F-tests for linear models and likelihood ratio tests for logistic models). False discovery rate (FDR) correction using the Benjamini–Hochberg procedure was applied to nested model comparisons and interaction analyses.

All tests were two-sided, and statistical significance was defined as $p < 0.05$.

Results

Study I

A total of 47 children with asthma at age 7 years with available T2 biomarker data were included, together with 165 children without asthma serving as a control group. Based on measurements of blood eosinophils, FeNO, and aeroallergen sensitization at age 7, asthma cases were classified as T2-high (n = 26) or T2-low (n = 21). Detailed study design, phenotype definition, and participant flow are presented in the appended manuscript (Study I).

Children with T2-high asthma displayed a markedly more atopic disease profile than those with T2-low asthma, including a higher prevalence of AR (76.9% vs. 19.0%) and atopic dermatitis (80.8% vs. 42.8%) during childhood, as well as higher total IgE levels (mean 87 vs. 20 IU/mL). Asthma in the T2-high group was characterized by a later age of onset (median 3.6 vs. 1.8 years, p = 0.008) and a longer disease duration (median 10.2 vs. 5.6 years, p = 0.019). By age 18 years, persistent asthma was present in 46.2% of children with T2-high asthma compared with 9.2% in the T2-low group (OR 8.14 [95% CI 1.57–42.34]).

Longitudinal lung function analyses showed that sRaw was 12.5% higher across childhood and adolescence in children with T2-high asthma compared with those with T2-low asthma (LMM estimate 0.53 [0.06–1.01], p = 0.029) (Figure 2).

Figure 2

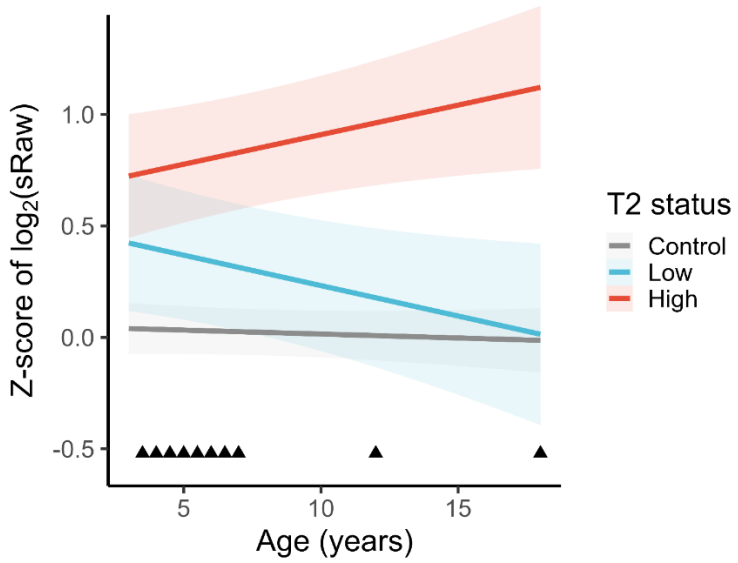


Figure 2. Longitudinal development of specific airway resistance (sRaw) by T2 phenotype. Model-estimated Z-scores of log_2(sRaw) from 3 to 18 years in adolescents with T2-high asthma (n=26), T2-low asthma (n=21), and controls (n=165). Z-scores were standardized using the control group (mean and SD). Shaded areas show 95% confidence intervals. Triangles indicate the ages at which sRaw was assessed. *Figure 2 corresponds to Figure 3 in the appended Study I manuscript.*

In contrast, no significant differences were observed between the two phenotypes for spirometric indices, including FEV_1 (estimate −0.24 [−0.58; 0.09], p = 0.16), MMEF (estimate −0.19 [−0.62; 0.24], p = 0.37), or the FEV_1/FVC ratio (estimate 0.03 [−0.06; 0.01], p = 0.12).

Children with T2-high asthma were more likely to exhibit bronchodilator reversibility, both when assessed by spirometry (OR 3.37 [1.03–11.00], p = 0.044) and by sRaw measurements (OR 2.60 [1.17–5.75], p = 0.019). Furthermore, airway hyperresponsiveness to methacholine (PD_{20}) was significantly increased in T2-high asthma compared with T2-low asthma (estimate −0.70 [−1.18; −0.23], p = 0.004). No differences were observed between the phenotypes for exercise-induced bronchoconstriction (p = 0.54) or cold-air responsiveness (p = 0.61).

The number of severe asthma exacerbations from early childhood to age 18 years did not differ between children with T2-high and T2-low asthma (median 1 vs. 2 exacerbations, $p = 0.87$).

Study II

Of the 176 children diagnosed with asthma during childhood, 163 had onset before age 17 years and 146 were subsequently confirmed by objective ERS criteria. Among these, 92 had ongoing asthma beyond age 17 years and were classified as having persistent asthma, whereas 54 no longer met diagnostic criteria and were considered to have remission. Children who did not develop asthma at any point during follow-up constituted the control group (n = 165).

For AR, 31 children fulfilled criteria for persistent disease, defined by presence at both 7 and 18 years. Teen-onset AR was observed in 90 children who were unaffected at 7 years but fulfilled criteria at 13 and/or 18 years. The comparison group consisted of children who never met criteria for AR or non-AR (n = 93).

Model performance differed across outcomes (Figure 3). Discrimination was highest for persistent asthma versus controls, where the Random Forest model achieved an AUC of 0.74. LightGBM demonstrated comparable performance. In contrast, separation of persistent asthma from asthma with remission was limited, with the best-performing model reaching an AUC of 0.64.

Prediction of AR trajectories showed weaker discrimination. For persistent AR versus controls, the highest AUC was 0.59 using Elastic Net. No model was able to distinguish teen-onset rhinitis from controls, with AUC values at or below 0.49. Overall, three of the four prediction tasks yielded discrimination close to chance.

Figure 3

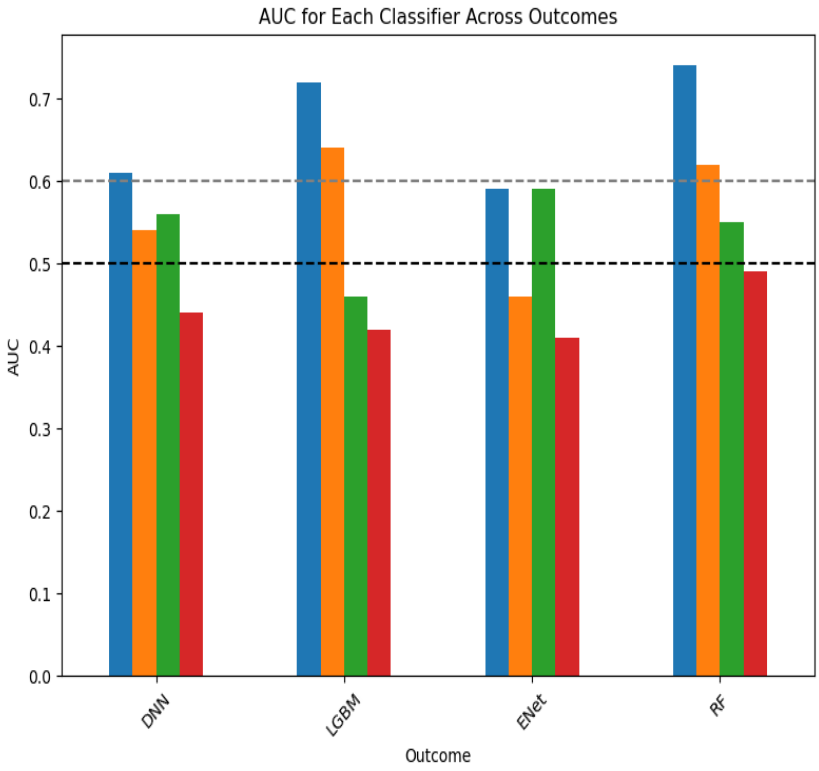


Figure 3. Discriminative performance across models and outcomes (Study II).
Area under the receiver operating characteristic curve (AUC) in the independent test set (40% hold-out) for four classifiers: deep neural network (DNN), LightGBM (LGBM), Elastic Net (ENet), and random forest (RF). Bars show AUC for four binary classification tasks: persistent asthma vs controls (blue), persistent asthma vs asthma with remission (orange), persistent allergic rhinitis (AR) vs controls (green), and teen-onset AR vs controls (red). The dashed lines indicate AUC = 0.5 (chance level) and AUC = 0.6 (threshold for "fair" discrimination). *This figure corresponds to Figure 1 in the appended Study II manuscript.*

Feature contribution patterns differed between outcomes. In the comparison of persistent asthma and controls, the most influential predictor in the Random Forest model was the cumulative level of aeroallergen-specific IgE at 6 years (Figure 4). Higher values contributed positively to the predicted probability of persistence. Additional variables with substantial influence included maternal age, neonatal expiratory flow, genetic risk score for asthma, inflammatory markers in infancy, gestational age, indoor allergen exposure, early-life eosinophil counts, and early environmental NO_2 levels. Two predictors - indoor humidity at 1 year and IL-6 at 6 months - showed non-linear relationships with model output.

Figure 4

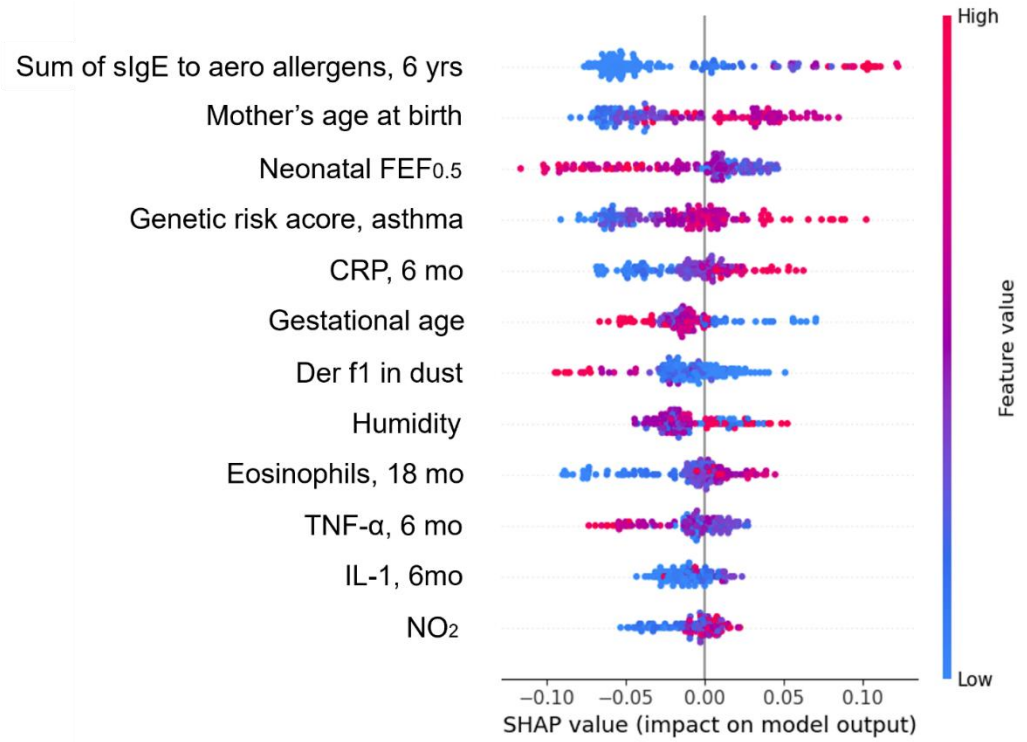


Figure 4. SHAP summary plot for the Random Forest model predicting persistent asthma versus controls.
Each point represents an individual child and shows the SHAP value for a given feature, reflecting its contribution to the predicted probability of persistent asthma. Positive SHAP values indicate increased predicted risk, whereas negative values indicate decreased risk. Features are ranked according to overall importance (mean absolute SHAP value). Color represents the feature value (red = high, blue = low). The cumulative level of aeroallergen-specific IgE at 6 years was the most influential predictor. *This figure corresponds to Figure 2 in the appended manuscript (Study II).*

When comparing persistent asthma with remission, total IgE at 4 years contributed most strongly to predictions in the LightGBM model. Lung function at 7 years, early airway infections, sibling number, and indoor dog allergen levels also influenced model output. Higher MMEF, more infections in early life, and more older siblings were associated with lower predicted probability of persistent disease.

For persistent AR versus controls, paternal history of AR had the greatest contribution to model predictions in the Elastic Net analysis. Other contributing variables included maternal atopy, socioeconomic indicators, early-life infection burden, antibiotic exposure in infancy, airway resistance and eosinophil levels in childhood, BHR, lung function measures, indoor allergen exposure, and early inflammatory markers.

We explored whether certain early-life factors only influenced disease persistence in combination with other factors. However, we did not identify consistent patterns indicating strong interaction effects. Instead, the models appeared to rely primarily on the individual contribution of each predictor.

Study III

At 18 years of age, 347 adolescents participated in the follow-up examination and had available sensitization data. Overall, 21% were sensitized to at least one pet allergen component. Sensitization was predominantly monosensitization, most commonly to Fel d 1 (18% of the cohort; 86% of sensitized individuals), whereas polysensitization was less frequent (36% of sensitized) and heterogeneous in pattern (Figure 5)

Figure 5

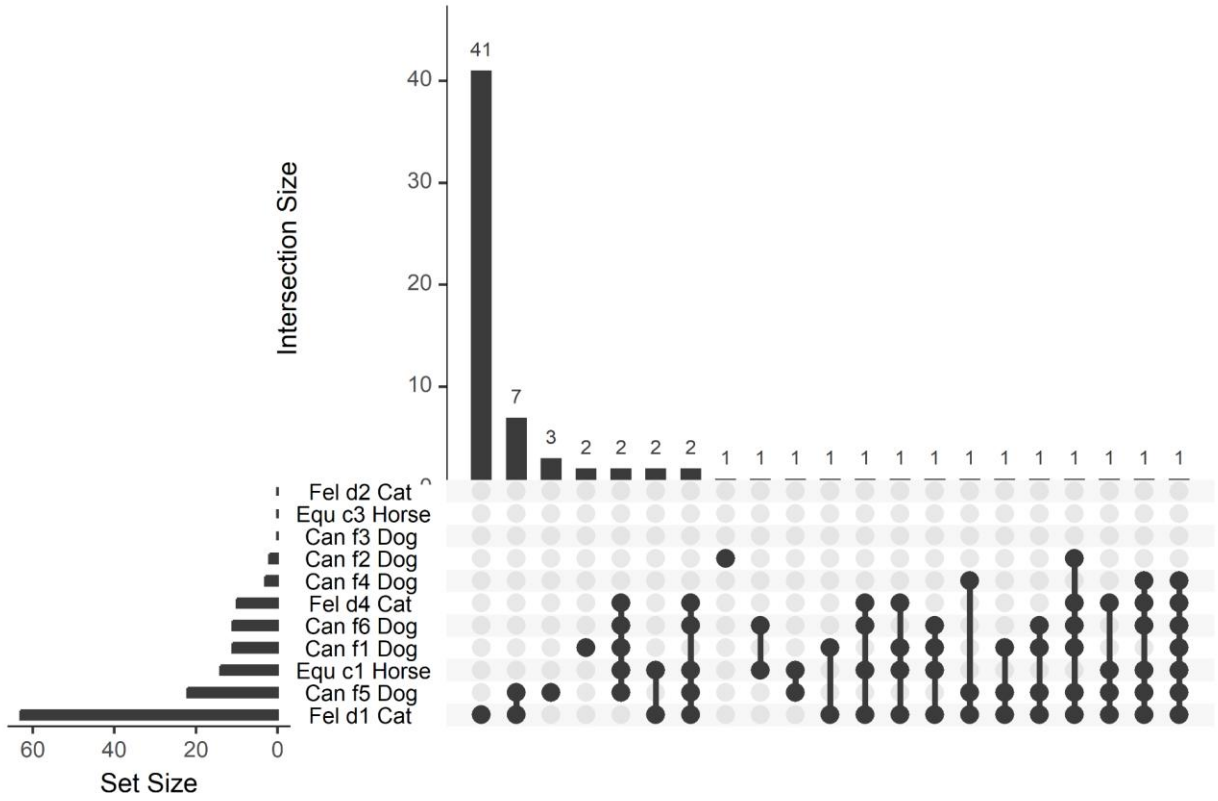


Figure 5. Co-sensitization patterns to pet allergen components at 18 years.

UpSet plot illustrating overlap between IgE sensitization to individual pet allergen components measured by ImmunoCAP ISAC. Bars represent the number of adolescents sensitized to each unique combination of components (intersection size), while horizontal bars indicate the total number sensitized to each individual component (set size). Monosensitization to Fel d 1 was the most frequent pattern (n = 41), whereas other sensitization patterns were less common and heterogeneous. *This figure corresponds to Figure 1 in the appended Study III manuscript.*

Whole-extract sensitization to cat (32.6% vs 13.2%, $p < 0.001$) and dog (34.8% vs 12.0%, $p < 0.001$) was significantly more frequent among adolescents with current asthma compared with those without asthma. Similarly, sensitization to the major molecular components Fel d 1 (30.3% vs 14.0%, $p = 0.001$), Can f 1 (10.1% vs 0.8%, $p < 0.001$), and Can f 5 (14.6% vs 3.7%, $p = 0.002$) was more frequent among adolescents with current asthma (Table III-2).

In multivariable regression analyses adjusted for smoking, pet exposure, and HDM sensitization, pet sensitization was not associated with baseline spirometric indices (FEV_1, FEV_1/FVC, MMEF) or sRaw (Figure 6A–B; Supplementary Table S1, Study III).

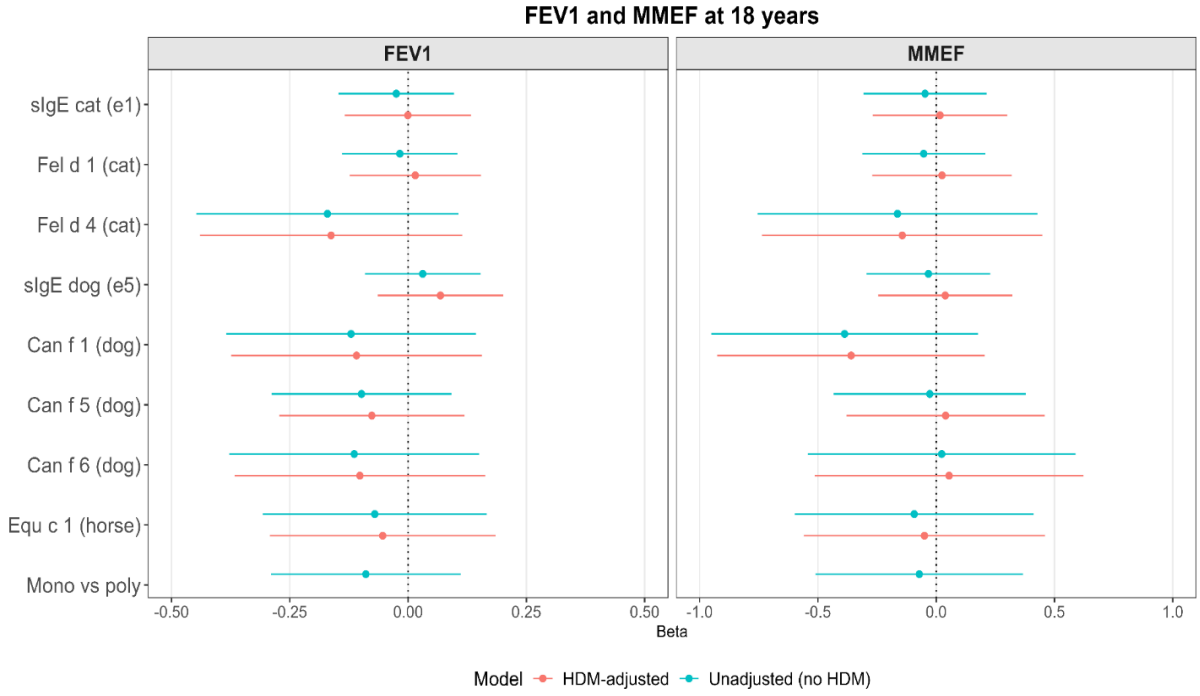
In contrast, sensitization was consistently associated with airway inflammation. After HDM adjustment, whole-extract sensitization to cat and dog remained associated with elevated FeNO (cat: 1.39-fold [1.15–1.69], $p < 0.001$; dog: 1.42-fold [1.17–1.72], $p < 0.001$). At the molecular level, Fel d 4 was associated with an 89% higher FeNO (1.89 [1.26–2.83], $p = 0.002$), Can f 6 with an 89% higher FeNO (1.89 [1.29–2.78], $p = 0.001$), and Equ c 1 with a twofold higher FeNO (2.00 [1.42–2.82], $p < 0.001$) (Figure 6B; Supplementary Table S1, Study III).

Similarly, several sensitizations were associated with increased bronchial hyperresponsiveness. After HDM adjustment, cat sIgE was associated with lower PD_{20} (0.54 [0.32–0.89], $p = 0.02$), and significant associations were observed for Can f 6 (0.23 [0.07–0.82], $p = 0.02$) and Equ c 1 (0.22 [0.08–0.60], $p = 0.003$), indicating enhanced airway responsiveness (Figure 6B; Supplementary Table S1, Study III).

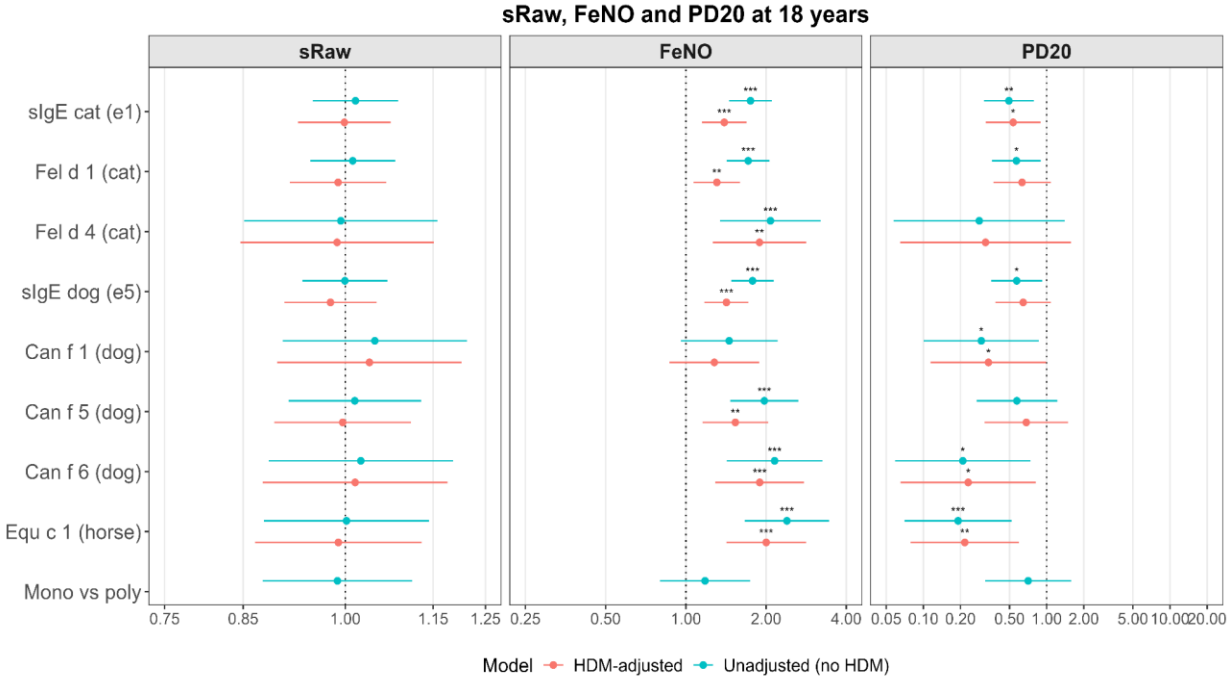
Whole-extract sensitization was also associated with current asthma (cat: OR 2.44 [1.28–4.67], $p = 0.01$; dog: OR 3.34 [1.76–6.38], $p < 0.001$). Among molecular components, Can f 1 demonstrated the strongest association (OR 11.94 [2.95–80.18], $p = 0.002$), whereas associations for cat components were attenuated after HDM adjustment (Figure 6C; Supplementary Table S1, Study III).

Figure 6. Associations between pet sensitization and objective airway outcomes at 18 years.

(A)



(B)



(C)

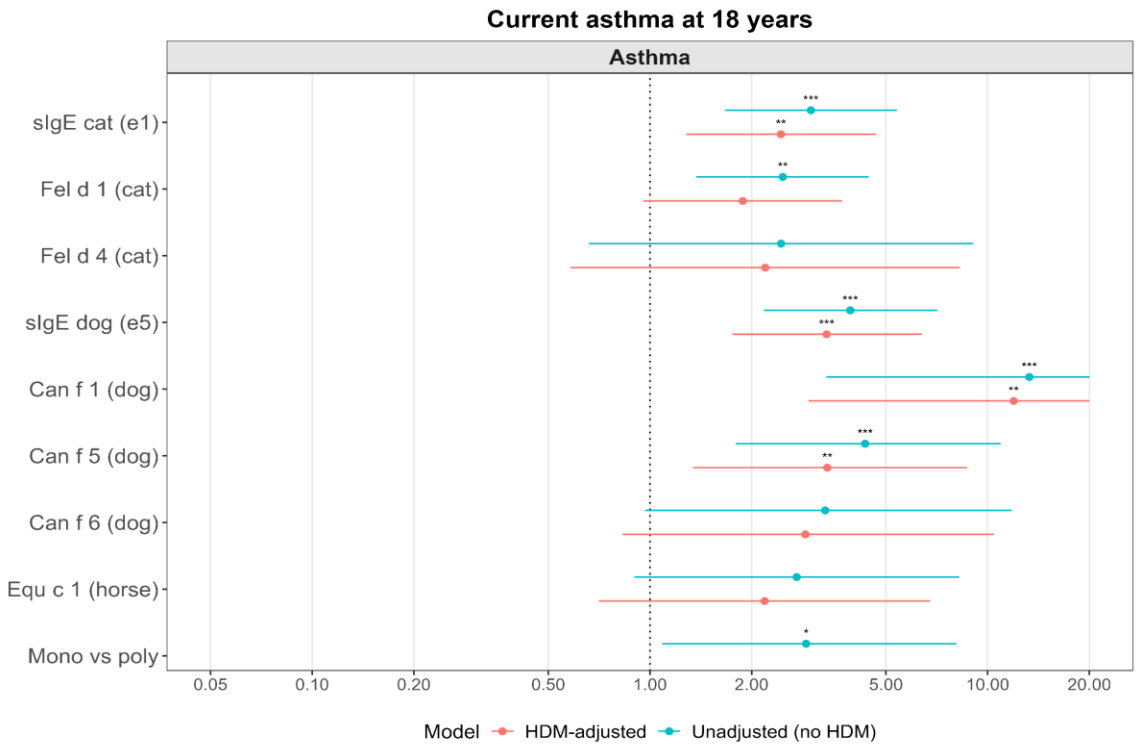


Figure 6. Associations between pet sensitization and objective airway outcomes at 18 years. Forest plots show regression estimates with 95% confidence intervals for whole-extract sensitization (cat sIgE e1; dog sIgE e5) and selected pet allergen components (ISAC), as well as monosensitization versus polysensitization. **(A)** Calibrated spirometric outcomes (FEV_1 and MMEF) are presented as β-coefficients. **(B)** Specific airway resistance (sRaw), fractional exhaled nitric oxide (FeNO), and methacholine PD_{20} are presented as fold-changes from log_2-transformed models. **(C)** Current asthma is presented as odds ratios. The vertical reference line indicates no association (β = 0 in panel A; ratio/OR = 1 in panels B–C). Points represent model estimates and horizontal lines 95% CIs. Models were adjusted for current smoking and relevant current pet exposure; HDM-adjusted models additionally included sensitization to house dust mite (Dermatophagoides pteronyssinus, d1). Sensitization was defined as sIgE ≥0.35 kUA/L for whole extracts and ISAC ≥0.30 ISU for components. *p<0.05; **p<0.01; ***p<0.001. *This figure corresponds to Figure 3A-C in the appended Study III manuscript.*

Polysensitized adolescents had higher odds of current asthma compared with monosensitized individuals (OR 2.90 [1.09–8.10], p = 0.04), but no consistent differences were observed for lung function, FeNO, or PD_{20} (see Supplementary Table S1, Study III).

Continuous ISAC analyses yielded directionally consistent but generally attenuated estimates (see Supplementary Table S2, Study III). Stratified analyses according to asthma status demonstrated similar patterns, although estimates were less precise in the asthma subgroup (see Supplementary Tables S3–S4, Study III). Formal interaction analyses did not reveal consistent effect modification by asthma status (see Supplementary Table S5, Study III).

Nested model comparisons showed no consistent incremental explanatory value of molecular component sensitization beyond whole-extract sIgE after correction for multiple testing (all FDR-adjusted q-values ≥ 0.09) (see Supplementary Table S6, Study III).

Discussion

Primary findings

In **Study I**, children with T2-high asthma had later disease onset, longer disease duration, and a significantly higher likelihood of asthma persisting into late adolescence compared with children with T2-low asthma. Across childhood and adolescence, T2-high asthma was characterized by higher airway resistance, greater BHR, and more bronchodilator reversibility. In contrast, conventional spirometric indices and the frequency of exacerbations did not differ between the groups. These findings indicate that T2-high asthma represents a more persistent phenotype in which airway inflammation and airway dysfunction are more discriminative than baseline spirometry.

In **Study II**, we found that early-life data had limited ability to predict long-term asthma and AR outcomes. Despite inclusion of a broad range of early-life variables, predictive performance was modest across outcomes. No single early-life factor, nor consistent combinations of factors, clearly distinguished persistent disease from other trajectories. Overall, these findings indicate that early-life measurements alone were insufficient to provide robust long-term prediction of disease course in this setting.

In **Study III**, we found that pet component sensitization at 18 years was dominated by monosensitization, most often to the cat component Fel d 1, while polysensitization was less frequent and heterogeneous. Pet sensitization - both whole-extract and component-resolved - was associated with current asthma, more airway inflammation, and increased BHR, but not with baseline lung function. Component-resolved diagnostics did not provide consistent incremental explanatory value beyond whole-extract sensitization in relation to objective airway outcomes.

Strength and limitations

A major strength of this thesis is the use of the $COPSAC_{2000}$ cohort, which provides prospective follow-up from birth with a very high level of clinical consistency and objective phenotyping. Children were examined at scheduled visits and during acute episodes by COPSAC physicians,

enabling precise characterization of disease onset, remission, and persistence across childhood and adolescence. Asthma and AR were defined using predefined algorithms incorporating symptoms, objective measurements, and treatment response, reducing misclassification and strengthening internal validity.

The longitudinal design with repeated objective assessments - including lung function, airway responsiveness, airway inflammation, and allergic sensitization - allowed evaluation of disease trajectories and clinically relevant endotypes rather than relying on cross-sectional snapshots. Importantly, the rich covariate information enabled adjustment for key clinical and environmental factors, supporting more specific interpretation of associations across the studies.

In Study II, a particular strength was the evaluation of a broad range of early-life clinical, immunological, environmental, and genetic factors within the same well-characterized cohort. This allowed a comprehensive assessment of whether information available in early childhood has meaningful prognostic value. The use of separate training and test sets reduced the risk of overly optimistic prediction estimates.

Some limitations should be acknowledged. All studies are observational, meaning that identified associations cannot be interpreted as proof of causality and may be influenced by factors not fully captured in the dataset. Although the cohort size (n = 411) is large for a deeply phenotyped clinical birth cohort, it remains modest for subgroup analyses and prediction modelling. This may reduce precision, particularly for rarer phenotypes and less prevalent sensitization patterns. In addition, limited numbers in certain exposure or outcome categories across the studies may have reduced statistical power.

The cohort consists of children born to mothers with asthma and includes limited ethnic diversity. While this design strengthens the ability to study mechanisms in a high-risk population, it may limit generalizability to broader populations.

Finally, treatment patterns may have influenced several outcomes. A substantial proportion of adolescents with asthma were treated with ICS at follow-up, which may have attenuated markers of T2 inflammation, BHR, and potentially lung function measures (16,63). This may have

reduced observable differences between phenotypes and limited separability in prediction analyses.

Interpretation

Across this thesis, we investigated heterogeneity and long-term trajectories of asthma and AR from childhood to early adulthood in the deeply phenotyped $COPSAC_{2000}$ cohort. A central finding was that a biomarker-defined T2-high endotype in childhood identified a subgroup characterized by later disease onset, longer duration, increased airway resistance, greater BHR, and a significantly higher likelihood of asthma persisting into late adolescence. In contrast, early-life characteristics more broadly showed limited ability to discriminate long-term disease trajectories at the individual level.

Current literature describes T2-high asthma as an endotype characterized by eosinophilic inflammation and allergic sensitization, with biomarkers such as FeNO, blood eosinophils, and aeroallergen sensitization reflecting ongoing type 2 immune activity (9,11–14). In this pediatric cohort, this biomarker-based definition was accompanied by a distinct and consistent physiological pattern across follow-up. Children classified as T2-high at age 7 exhibited persistently higher sRaw, greater BHR, and more bronchodilator reversibility than those with T2-low asthma. In contrast, conventional spirometric indices and exacerbation rates did not differ substantially between groups.

Thus, children classified as T2-high represented a clinically distinct subgroup with a significantly higher likelihood of asthma persisting into late adolescence. This aligns with longitudinal cohort studies demonstrating that early allergic sensitization and BHR are associated with an increased risk of persistent asthma into adolescence (3,5,64–66), even when baseline spirometric impairment is not pronounced (3,37). Our findings extend these observations by showing that this pattern is already identifiable in childhood and remains evident across adolescence.

T2 inflammatory activity was primarily reflected in measures of airway resistance and BHR rather than in conventional spirometric indices. Children with a more persistent asthma course were characterized by higher sRaw and greater BHR, whereas FEV_1 and flow measures did not

meaningfully distinguish disease trajectories. These findings are consistent with previous studies suggesting that spirometry may remain within the normal range despite ongoing inflammatory activity and airway dysfunction (3,37,55,63,67,68). Clinically, this indicates that spirometry alone may not adequately capture airway abnormalities in children with a T2-high phenotype. Incorporating measures of airway responsiveness and inflammatory biomarkers may therefore provide complementary information when characterizing disease activity and heterogeneity in pediatric asthma.

Study III supports the same overall pattern from a cross-sectional perspective. In adolescence, sensitization to pet allergens was associated with elevated FeNO and increased BHR, but not with clear differences in baseline spirometry. In addition, pet allergen sensitization was associated with current asthma, linking T2-related sensitization not only to airway physiology but also to clinical disease status.

From a biological perspective, sustained T2 immune activation - reflected by IgE sensitization, eosinophilia, and elevated FeNO - characterized a subgroup in whom airway resistance and BHR were consistently more pronounced. In Study II, allergic sensitization likewise emerged as a key predictive signal: the sum of sIgE to aeroallergens at 6 years was the most influential feature for discriminating persistent asthma from controls in the best-performing model. This suggests that sensitization appeared both as a defining feature of a persistent inflammatory endotype and as one of the strongest early-life indicators of long-term disease risk.

Together, these findings position allergic sensitization as a central clinical marker across analyses - associated with measurable differences in airway physiology (Studies I and III) and informative for long-term asthma risk stratification (Study II). While the present work does not address temporal or causal relationships between sensitization, inflammation, and airway responsiveness, the consistent appearance of sensitization across both physiological and predictive frameworks underscores its relevance when characterizing pediatric asthma phenotypes and trajectories.

Regarding prediction, a systematic review of clinical prediction models found no consistent performance advantage of machine learning over logistic regression in comparisons at low risk of bias, and highlighted substantial methodological limitations in many published studies (69).

Consistent with these observations, our analyses did not show a clear performance advantage of more flexible machine learning models, nor did we identify robust interaction patterns that materially improved discrimination. The limited discrimination observed - particularly for remission versus persistence and for AR trajectories - may reflect both the inherent complexity of disease trajectories and the substantial data requirements of flexible modeling approaches, suggesting that early-life predictors alone incompletely capture the dynamic processes shaping long-term outcomes.

In pet allergy, CRD provided a more detailed characterization of sensitization patterns but did not consistently improve explanatory value beyond whole-extract sensitization in relation to airway physiology. In this adolescent population, whole-extract sensitization appeared to capture most of the airway-related signal at the group level, although molecular profiling may still be useful in selected clinical situations.

Overall, the thesis shows that in a high-risk birth cohort, T2 inflammatory activity in childhood identifies a subgroup with increased airway resistance, heightened BHR, and a substantially higher probability of persistent asthma into late adolescence. Across all three studies, inflammatory and sensitization-related markers were more closely aligned with disease persistence and airway reactivity than baseline spirometry, whereas early-life clinical and environmental characteristics alone provided limited prognostic precision. These findings underline the dynamic nature of pediatric asthma and the importance of integrating inflammatory and physiological assessments when characterizing disease trajectories over time.

Conclusion

The overall aim of this thesis was to investigate the development, heterogeneity, and long-term outcomes of asthma and AR from childhood to early adulthood in the $COPSAC_{2000}$ birth cohort, with particular focus on T2 inflammatory phenotypes, early-life predictors of disease persistence, and molecular patterns of allergic sensitization.

The findings in the thesis demonstrate that T2 inflammatory activity and allergic sensitization are consistently associated with airway dysfunction and persistence of asthma into late adolescence, whereas early-life clinical and environmental characteristics alone provide limited prognostic precision. Across studies, inflammatory and sensitization-related markers were more closely aligned with disease persistence and airway reactivity than baseline spirometric measures.

Study I

Aim: To characterize T2-high and T2-low asthma phenotypes and their clinical and physiological trajectories across childhood.

In this longitudinal birth cohort, we characterized T2-high and T2-low asthma at age 7 years and examined their clinical and physiological trajectories across childhood and adolescence. Asthma classified as T2-high identified a clinically distinct subgroup with later disease onset, longer duration, and a significantly higher likelihood of persistent asthma into late adolescence compared with T2-low asthma.

Across follow-up, T2-high asthma was associated with higher sRaw, greater bronchial BHR, and more frequent bronchodilator reversibility, indicating sustained airway dysfunction. In contrast, conventional spirometric indices, including FEV_1, and exacerbation rates did not meaningfully differ between the phenotypes.

Overall, these findings demonstrate that T2 inflammatory activity in childhood is linked to a more persistent asthma trajectory characterized by increased airway resistance and BHR, whereas baseline spirometry alone does not adequately distinguish long-term disease course.

Study II

Aim: To evaluate the potential of early-life data to predict persistent asthma and AR using machine learning approaches.

In this study, we evaluated the potential of early-life data to predict persistent asthma and AR trajectories into adolescence using explainable machine learning models. Despite applying multiple algorithms, including Random Forest, LightGBM, Elastic Net, and Deep Neural Network, predictive performance was limited across outcomes.

Only the comparison between persistent asthma and controls reached acceptable discrimination, with the highest AUC of 0.74 observed for the Random Forest model and comparable performance for LightGBM. In contrast, discrimination between persistent and remitting asthma, and for AR trajectories, was modest and often close to chance. Importantly, SHAP analyses did not reveal consistent or meaningful interaction patterns between predictors.

Although allergic sensitization, particularly cumulative aeroallergen sIgE, emerged as an influential predictor of persistent asthma, no robust combinations of early-life clinical, immunological, genetic, and environmental factors reliably distinguished long-term disease trajectories at the individual level.

Overall, these findings suggest that remission and persistence of asthma and AR cannot be robustly determined from early-life data alone. This may reflect limited information content in early childhood measures, constraints in statistical power for detecting complex interactions, or the possibility that long-term disease trajectories are shaped by later-life exposures and partly stochastic processes.

Study III

Aim: To examine molecular patterns of allergic sensitization and their associations with lung function and airway inflammation in adolescence.

At 18 years of age, pet allergen sensitization was predominantly characterized by monosensitization, most commonly to the cat component Fel d 1. Both whole-extract and molecular sensitization were consistently associated with increased airway inflammation, enhanced BHR, and current asthma. In contrast, no consistent associations were observed with baseline spirometric indices or sRaw.

Molecular component sensitization did not provide consistent incremental explanatory value beyond whole-extract specific IgE in relation to lung function, airway inflammation, BHR, or current asthma after correction for multiple testing. Thus, while component-resolved diagnostics refined the characterization of sensitization patterns, they did not substantially improve the assessment of objective airway outcomes in this adolescent population.

Future perspectives

This thesis supports that asthma and AR should be understood as dynamic, heterogenic conditions that evolve across childhood and adolescence, and that their clinical expression is not fully captured by single time-point classifications. Across the three studies, inflammatory and sensitization-related markers were repeatedly linked to BHR and disease persistence, whereas baseline spirometric indices were less consistently discriminative. Together, these findings underline the need for future research to move beyond static definitions and instead focus on how repeated objective measurements can improve characterization, monitoring, and risk assessment over time. Ultimately, this may support a more differentiated clinical follow-up, where children at higher risk of persistent disease and ongoing airway dysfunction are identified earlier and monitored more closely, while others may be followed with a less intensive approach.

A central next step is to determine whether the biomarker-based stratification applied in Study I is reproducible outside the $COPSAC_{2000}$ setting. The T2-high definition identified a subgroup with higher sRaw, greater BHR, and a significantly higher likelihood of asthma persistence into late adolescence. Future studies should validate similar phenotype definitions in independent and population-based cohorts, and evaluate whether the same biomarker thresholds perform consistently across populations with different baseline risk, environmental exposures, and treatment patterns. Such work should also examine stability and transitions of inflammatory profiles across follow-up, as children may change phenotype as sensitization develops, symptoms fluctuate, and treatment is introduced. A key question is whether repeated inflammatory profiling improves the ability to identify children whose disease will persist, compared with a single biomarker assessment at one age. If confirmed, such stratification could contribute to more individualized follow-up strategies in pediatric asthma.

Regarding prediction, the findings from Study II highlight that early-life information alone was insufficient to provide robust discrimination between long-term asthma and AR trajectories - particularly for remission versus persistence and for AR outcomes. This suggests that important determinants of later disease course may emerge after early childhood, and that future prediction research should focus on approaches that incorporate information accumulated during follow-up

rather than relying solely on the early-life period. Future studies should therefore evaluate models that update risk estimates at clinically relevant timepoints, integrating repeated measurements of sensitization, FeNO, symptom burden, treatment patterns, and objective physiology. In addition, the field would benefit from larger, collaborative datasets enabling external validation and evaluation of generalizability across cohorts. Future work should also clarify whether such prediction models can meaningfully inform clinical decision-making during longitudinal follow-up.

With regard to allergic sensitization, this thesis shows that molecular component testing did not provide consistent incremental explanatory value beyond whole-extract sensitization in relation to airway physiology at 18 years. However, interpretation is limited by the relatively low prevalence of several individual components, resulting in limited statistical power for detecting component-specific effects. Future studies should clarify whether CRD are more informative in larger population-based cohorts with adequate representation of component-sensitized individuals. While studies in severe asthma populations have suggested greater clinical relevance of molecular sensitization patterns, it remains to be determined whether such findings translate to milder or general population settings. Larger studies with sufficient numbers of polysensitized individuals are needed to determine whether molecular patterns contribute to risk stratification beyond conventional testing. These investigations should explicitly evaluate whether molecular profiling changes clinical interpretation or management.

Finally, the consistent association between inflammatory markers and BHR, in the absence of clear differences in baseline spirometry, suggests that conventional spirometric indices may not fully capture airway dysfunction in treated adolescent populations. Future longitudinal studies should continue to integrate measures such as sRaw and BHR alongside spirometry to improve physiological characterization of asthma across developmental stages. Such work may help refine objective assessment strategies across childhood and adolescence.

As such, risk stratification in pediatric asthma and AR will require replication across cohorts, incorporation of repeated objective measurements during follow-up, and careful evaluation of which biomarkers provide clinically meaningful information beyond established tools. Such

work may ultimately support more individualized monitoring and management strategies throughout childhood and adolescence.

Summary

Asthma and allergy are among the most common chronic diseases in childhood and have a significant impact on the children's quality of life and health. Despite extensive research, the development of these "atopic diseases" and their progression remain incompletely understood. Why some children outgrow their asthma and allergy while others develop a more severe and persistent course remains an unanswered question.

We know from previous research that the risk of developing persistent asthma and allergies in childhood is shaped by a complex interplay of genetic predisposition, environmental exposures, infection history, and immune system maturation - often characterized by considerable individual variability. Furthermore, increasing attention has also been directed toward identifying and characterizing distinct asthma phenotypes, as disease progression and treatment response are likely to differ according to the underlying inflammatory profile. Asthma is frequently classified based on immunological patterns, with particular focus on type 2 (T2) inflammation - an immune response closely associated with allergic sensitization and eosinophilic activation. So-called "T2-high" asthma is typically linked to a higher disease burden and more severe outcomes in adults, but knowledge regarding the clinical significance of the phenotype in childhood remains limited.

A new technology in allergy diagnostics, component resolved diagnostics (CRD), has enabled a more in-depth understanding of allergic sensitization. With this technique, it is possible to study sensitization patterns and investigate whether specific allergen components, such as those from cats, dogs or horses, may be more closely associated with disease development and impaired lung function than previously assumed.

Aim:
The aim of this thesis is to contribute to an understanding of how environment, genetics, exposures, and biomarkers in childhood relate to the development and persistence of asthma and allergic rhinitis—from early childhood to adolescence. The projects aim to examine how T2-inflammatory phenotypes manifest in children and how early-life factors and biological markers may help predict disease progression. Furthermore, the importance of sensitization to specific

allergen components is investigated, and whether these can be indicators of reduced lung function and a more severe disease course.

Hypotheses:

- The type of inflammatory response and the severity of asthma (T2-low and T2-high asthma).
- Early life factors in childhood (ages 0–7) play an important role in the development of atopic disease.
- Sensitization to specific components from furry animals (dog, cat, and horse) influences lung function and thus the severity of potential asthma.

Study Design:

The thesis is based on data from the birth cohort, Copenhagen Prospective Studies on Asthma in $Childhood_{2000}$ ($COPSAC_{2000}$), which includes 411 children born to mothers with asthma. The children have been closely followed with regular clinical visits from birth through childhood and adolescence, with systematic collection of data on symptoms, environmental exposures, biomarkers, genetics, lung function, and allergic sensitization.

Clinical Relevance:

The aim is to contribute new knowledge that can help with a more differentiated approach to risk assessment and treatment. The objective is to investigate the relationships between asthma phenotypes, allergic sensitization, and the disease course of asthma and allergic rhinitis in order to identify children at increased risk of a more severe and persistent disease progression.

Dansk resumé

Astma og allergi er nogle af de hyppigste kroniske sygdomme i barndommen og har stor betydning for børnenes livskvalitet og helbred. Trods meget forskning er udviklingen af disse ”atopiske sygdomme” og deres årsag og forløb stadig ikke fuldt afklaret. Hvorfor nogle børn vokser fra deres sygdom, og andre udvikler et sværere og vedvarende forløb, er fortsat et ubesvaret spørgsmål.

Fra forskning ved vi, at børns risiko for at udvikle vedvarende astma og allergi formes af et komplekst samspil mellem genetiske faktorer, miljøpåvirkninger, infektionseksponering og immunmodning – ofte præget af betydelig individuel variation. I de senere år har der desuden været stigende fokus på at identificere og karakterisere forskellige astmafænotyper, da sygdommens forløb og behandlingsrespons sandsynligvis varierer afhængigt af den underliggende inflammatoriske profil. Astma klassificeres ofte ud fra immunologiske mønstre, hvor særlig opmærksomhed er rettet mod type 2 (T2)-inflammation – en inflammatorisk respons tæt forbundet med allergisk sensibilisering og eosinofil aktivering. Den såkaldte "T2-high" astma er typisk associeret med øget sygdomsbyrde og sværere forløb hos voksne, men viden om fænotypernes betydning i barnealderen er fortsat begrænset.

En ny teknologi indenfor allergidiagnostik er component resolved diagnostics (CRD), og den har muliggjort en mere indgående forståelse af allergisk sensibilisering. Med denne teknik kan man undersøge sensibiliseringsmønstre og undersøge, om bestemte allergenkomponenter, fra fx kat, hund og hest, måske er tættere knyttet til sygdomsudvikling og nedsat lungefunktion end tidligere antaget.

Formål

Formålet med denne afhandling er at bidrage til en forståelse af, hvordan miljø, genetik, eksponeringer og biomarkører i barndommen relaterer sig til udviklingen og persistensen af astma og allergisk rhinitis – fra tidlig barndom til ungdom. Projektet har til formål at undersøge, hvordan T2-inflammatoriske fænotyper manifesterer sig hos børn, og hvordan tidlige faktorer og biologiske markører måske kan prædiktere sygdomsforløbet. Derudover belyses betydningen af

sensibilisering over for specifikke allergenkomponenter, og om disse kan være indikatorer for nedsat lungefunktion og et mere alvorligt sygdomsforløb.

Hypoteser

- Der er sammenhæng mellem typen af inflammatorisk respons og sværhedsgraden af astma (såkaldt T2-low og T2-high asthma)
- Faktorer i den tidlige barndom (0-7 år) har afgørende betydning for udvikling af atopisk sygdom
- Sensibilisering over for bestemte allergen komponenter fra pelsdyr (hund, kat og hest) har betydning for lungefunktion og således sværhedsgraden af eventuel astma

Studie design

Tesen er baseret på data fra fødselskohorten, Copenhagen Prospective Studies on Asthma in Childhood$_{2000}$ (COPSAC$_{2000}$), der består af 411 børn født af mødre med astma. Børnene er blevet fulgt tæt med regelmæssige kliniske besøg fra fødslen og op gennem barndommen og ungdommen, med registrering af symptomer, miljø, biomarkører, genetik, lungefunktion og allergisk sensibilisering.

Klinisk betydning

Det tilstræbes at bidrage med ny viden, der kan danne grundlag for en mere differentieret tilgang til risikovurdering. Formålet er at belyse sammenhænge mellem astma fænotyper, allergisk sensibilisering og sygdomsforløb af astma og allergisk rhinitis med henblik på at kunne identificere børn, der er i øget risiko for et mere alvorligt og vedvarende sygdomsforløb.

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Original articles

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ORIGINAL ARTICLE

WILEY

Type 2-high airway inflammation in childhood asthma distinguishes a more severe phenotype

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Abstract

Background: It remains unclear whether phenotyping of type 2-high (T2-high) asthma can distinguish clinical characteristics and lung function trajectories in childhood.

Objective: To explore differences between T2-high and T2-low asthma from birth to age 18 years.

Methods: We included 47 children with asthma and 165 as a control group from the Copenhagen Prospective Studies on Asthma in Childhood$_{2000}$ mother–child cohort. T2-high and T2-low asthma was defined at age 7 by sensitization to aeroallergens, elevated eosinophilic blood count, and/or elevated fractional nitric oxide. Lung function measurements included whole-body plethysmography, spirometry, exercise test, cold air provocation, and methacholine challenge. Differences in lung function trajectories and traits were analyzed using linear mixed models, Wilcoxon rank-sum test, Fisher's exact test, and Quasi-Poisson regression.

Results: At age 7 years, 47 had asthma (26 T2-high, 21 T2-low). By age 18, 12 (46.2%) with T2-high had persistent asthma whereas 2 (9.2%) with T2-low; OR 8.14 [1.57–42.34]. Specific airway resistance (sRaw) was 12.5% higher through childhood in children with T2-high asthma (estimate 0.53 [0.06; 1.01]); lung function was more reversible (OR 3.37 [1.03–11.00] for spirometry and OR 2.60 [1.17; 5.75] for sRaw), and they had increased airway hyperresponsiveness (AHR) to methacholine (as shown by 41% lower dose required to cause a 20% drop in lung function (estimate −0.70 [−1.18; −0.23])). There was no significant difference in exacerbation rate and other lung function measurements.

Conclusion: Childhood T2-high asthma differs from T2-low asthma in terms of onset, duration, airway resistance, and airway responsiveness.

Abbreviations: COPSAC$_{2000}$, COpenhagen Prospective Studies on Asthma in Childhood$_{2000}$; FEF50, Forced Expiratory Flow at 50% of FVC; FeNO, Fractional Exhaled Nitric Oxide; FEV0.5, Forced Expiratory Volume at 0.5 seconds; FEV1, Forced Expiratory Volume in 1 second; GLMM, Generalized linear mixed models; ICS, Inhaled Corticosteroids; LMM, Linear Mixed Model; PD15, Provocative Dose causing a 15% fall in FEV1; PD20, Provocative Dose causing a 20% fall in FEV1; SABA, Short-Acting Beta Agonists; sIgE, Specific Immunoglobulin E; SPT, Skin Prick Test.

KEYWORDS
asthma, eosinophils, longitudinal studies, pediatrics, phenotype

1 | INTRODUCTION

The most common asthma phenotype classification is based on the degree of T-helper cell 2 (Th2) inflammation, which is a major driver of asthma.[1] Type 2-high (T2-high) asthma is characterized by eosinophilic inflammation and the presence of Th2 cytokines, while T2-low asthma is characterized by neutrophilic inflammation and innate immune activation.[2,3] T2-high asthma is typically characterized by elevated fractional exhaled nitric oxide (FeNO) and blood eosinophil count, and allergic sensitization, while biomarkers for T2-low asthma remain poorly defined.

T2-high asthma has been associated with increased severity and higher exacerbation rates,[4–6] later onset,[5,7] greater persistence,[8] and lower pulmonary function.[5,7] In contrast, T2-low asthma presents a distinct clinical profile characterized by neutrophilic inflammation, higher body mass index,[9] reduced steroid responsiveness,[9–11] and less bronchodilator reversibility.[9] Emerging evidence further highlights that T2-high asthma represents a chronic and severe phenotype, driven by persistent eosinophilic inflammation and Th2 cytokine activity, which contributes to airway remodeling and greater disease burden over time.[12] However, most of the available evidence on T2-high and T2-low asthma is derived from studies in adult populations.[4,5,7,9–11] While some studies reference mixed cohorts, including children,[6,8] only few studies[12] have specifically focused on the pediatric T2 phenotype of asthma.

Despite extensive characterization of these phenotypes, the progression and outcomes of T2-high and T2-low asthma from childhood into adulthood has yet to be investigated. Current research focuses on cross-sectional analyses or short-term prospective studies, which do not fully capture the long-term impacts of these phenotypes. Additionally, there is a lack of comprehensive data integrating biomarkers and clinical symptoms over extended periods, which are crucial for understanding the multifactorial nature of asthma.

We aimed to explore differences between the T2-high and T2-low asthma phenotypes from birth to age 18 years in the Danish COpenhagen Prospective Studies on Asthma in Childhood$_{2000}$ (COPSAC$_{2000}$) at-risk birth cohort focusing on disease debut, persistence, exacerbations, and lung function trajectories.

Key message

Our study provides critical insights into the progression of T2-high versus T2-low asthma from childhood to adolescence, showing that T2-high asthma represents a more severe phenotype characterized by higher airway resistance, increased bronchial hyperresponsiveness, and greater persistence over time. This work is important as it highlights the need for early identification and targeted management of T2-high asthma in children to improve outcomes, advancing the understanding of asthma phenotypes and supporting more personalized treatment strategies.

2 | METHODS

2.1 | Study population

The COPSAC$_{2000}$ cohort is a single-center prospective mother–child cohort study. The cohort consists of 411 children born to mothers with a history of asthma between 1998 and 2001. The children were seen by COPSAC physicians every 6 months between ages 0–7 years and thereafter at 13 and 18 years. Additionally, children with acute troublesome lung symptoms were also seen and treated by physicians at the COPSAC clinic during the acute phase. Children were included in this study if they had asthma at age 7 years and concurrent measurements of sensitization to aeroallergens (assessed by sIgE and/or SPT), FeNO, and/or eosinophilic blood count. The control group consisted of children that had participated in at least one visit between 0 and 6.5 years of age and at the 7-, 13-, and 18-year visits and had no history of asthma. Children with asthma at ages other than 7 were excluded. Study design and rationale has been described previously.[13]

2.2 | Ethics

The Copenhagen Ethics Committee (KF 01–289/96) and The Danish Data Protection Agency (2015-41-3696) approved the study. Oral and written informed consent was obtained from both parents at enrollment.

2.3 | Asthma diagnosis

Asthma was rigorously defined and managed solely by COPSAC physicians, adhering to a detailed and validated algorithm. Asthma was diagnosed based on 4 comprehensive criteria that included: (1) recurrent episodes of troublesome lung symptoms defined as at least five episodes lasting three consecutive days within 6 months or daily symptoms for four consecutive weeks; (2) typical asthma symptoms such as prolonged nocturnal cough, exercise-induced symptoms, and symptoms causing nighttime awakenings; (3) response to intermittent use of inhaled β2-agonists; and (4) a positive response followed by a relapse after a three-month course of inhaled corticosteroids

(ICS). A relapse was identified by either two episodes of symptoms lasting 3 days within 3 months or symptoms occurring more than twice per week for two consecutive weeks. Severe acute symptoms or exacerbations requiring hospitalization or oral corticosteroids also led to an asthma diagnosis, initiating a six-month course of ICS treatment.[13,14]

Asthma remission was considered when there were no symptoms or need for asthma treatment for 1 year.[15]

2.4 | Asthma onset, duration, and persistence

Due to the strict algorithm, we have precise data on the asthma age of onset and remission. For this study, only children with a confirmed asthma diagnosis at age 7 were included; children who achieved asthma remission prior to this age were not part of the analysis. Yearly ICS treatment pauses were implemented if the patient had been asymptomatic for a minimum of 6 months. If the child remained asymptomatic for an additional 12 months following the discontinuation of ICS, the asthma diagnosis was considered in remission. Persistent asthma was defined as having an asthma diagnosis at 7 years and at the 18-year follow-up visit.

2.5 | T2-high/T2-low asthma definition

We classified asthma in 2 categories, T2-high and T2-low, based on the following biomarkers as previously described[16–18]:

- FeNO ≥20 ppb at age 7
- Blood eosinophil count $\geq 0.5 \times 10^9$/L at age 7
- Sensitization to aeroallergens (measured by skin prick test (SPT) and specific IgE (sIgE) levels) at age 7

If one or more of the criteria were met, the asthma was classified as T2-high, and if none of the criteria were met, the asthma was classified as T2-low. Mean blood eosinophil count showed great variability from age 0–18 years, and children with T2-high asthma had a significantly higher blood eosinophilic count as compared to both children with T2-low asthma, specifically 0.19×10^9 more eosinophilic blood cells per liter (0.19, [0.10–0.27], $p < .0001$). For both groups, blood eosinophil count peaked at 7 years of age (Figure S2). In a sensitivity analysis exploring the cutoff of blood eosinophil count, 45 out of the 47 asthma cases had T2-high asthma using a cutoff of 0.15×10^9/L, while a slightly higher cutoff of 0.3×10^9/L identified 38 T2-high asthma cases.

2.6 | Lung function

Neonatal lung function was measured at 1 month of age and was evaluated using a specialized technique known as the volume-anchored raised volume rapid thoracoabdominal compression technique, also referred to as the "squeeze jacket". This technique enabled the assessment of expiratory volume in the first 0.5 s (FEV0.5) and forced expiratory flow at 50% (FEF50) through spirometry measurements. Additionally, neonatal bronchial reactivity to methacholine was determined by estimating the dose required to induce a 15% reduction in transcutaneous oxygen pressure (PD15).[15,19]

Childhood lung function was assessed using spirometry measuring forced expiratory volume the first second (FEV1), forced vital capacity (FVC), and maximal mid-expiratory flow (MMEF), and whole-body plethysmography measuring specific airway resistance (sRaw). These measurements were obtained at every planned as well as acute visit from age 3 and 5 years for whole-body plethysmography and spirometry, respectively.[15,20–22]

Bronchodilator response was measured throughout the entire follow-up period at every planned visit from age 3 years to 5.5 years and additionally at asthma control visits every 6 months. Short-acting beta-2-agonist (SABA) (Bricanyl 0.25 mg per puff/Ventoline 0.1 mg per puff, AstraZeneca) was administered after baseline lung function was measured. For reversibility testing, a standard dose of four puffs (total 0.4 mg Ventoline or 1.0 mg Bricanyl) was given, regardless of age. Lung function measurements were repeated 15 min. after the administration of SABA. The test was considered positive if FEV1 improved >12% and/or sRaw dropped >30%.[23]

Bronchial responsiveness to methacholine was estimated at ages 6.5, 13, and 18 years, measuring the methacholine dose that resulted in a 20% decrease in FEV1 (PD20).[15] Bronchial responsiveness to exercise was tested at age 7 years, measuring the maximum percentage decrease in FEV1 after running on a treadmill for a minimum of 4 min with a pulse over 80%–90% of the max pulse. If FEV1 dropped >10%, the test was considered positive.[23] Bronchial responsiveness to cold, dry air was measured at age 6 years. If lung function (FEV1) dropped >10%, the test was considered positive.[15]

2.7 | Exacerbations

Exacerbations were recorded prospectively and defined as acute, severe asthmatic symptoms that necessitated treatment with oral prednisolone (1 mg/kg) for 3 days or high-dose inhaled corticosteroid therapy (specifically, budesonide at 1600 µg/day for 14 days) or led to hospital admission.[14,24,25]

2.8 | Statistical methods

For atopic endpoints, differences between groups were assessed using Fisher's exact test due to the categorical nature of these data. Continuous outcomes that were not normally distributed, including the age of onset, asthma persistence, duration, exercise tests, and cold air provocation, were analyzed using the Wilcoxon

rank test. We employed Quasi-Poisson regression to examine the number of exacerbations through childhood. Total IgE levels were logarithmic transformed to obtain a normal distribution before using Welch Two Sample t-test, after which the results were converted back to the original scale. FEV1, MMEF, and sRaw were corrected for height, sex, age, and concurrent airway infection using regression models, with residuals centered around the mean. Furthermore, FEV1, MMEF, PD15/PD20, and sRaw were z-scored to facilitate comparisons, while the FEV1/FVC index was analyzed without z-scoring through linear mixed models (LMM). Children without asthma at age 0–18 years constituted the control group (Z-score = 0). Additionally, sRaw and PD20 were logarithmically transformed before calculating Z-scores to ensure normal distribution prior to analyses.[19,22,24]

To analyze eosinophilic blood count and lung function measurements over time, we used linear mixed models (LMM) and generalized linear mixed models (GLMM) to account for within-subject correlations and repeated measures. The models included both fixed and random effects. Fixed effects included main predictor variables such as asthma status or T2-high status, while random effects were used to account for the repeated measures on the same subjects over time. For lung function measurements (sRaw, FEV1, MMEF, FEV1/FVC, and methacholine challenge test) and eosinophilic blood count, we used LMM with random intercepts for subjects. For binary outcomes (reversibility testing), we used GLMM with a binomial family and logit link. For reversibility testing and methacholine challenge test, we included random intercepts for subjects and random slopes for age. Random slopes were included only if the log-likelihood ratio test indicated a significantly higher likelihood compared to simpler models with random intercepts only. Otherwise, the intercept-only model was chosen as the best fit. The log-likelihood test was used for all model comparisons.

To facilitate interpretation, estimates from the LMM for log-transformed lung function measurements (sRaw, FEV1, FVC, MMEF, and PD15/PD20) were back-transformed and expressed as percentage changes. GLMM was presented as odds ratio. Estimate for eosinophilic blood count was presented in absolute values.

To ensure data integrity, our analysis was performed only on complete case data, excluding missing values and without using imputation. All statistical analyses were conducted in R version 4.2.2, using a two-sided significance level of $p < .05$. LMM analysis was performed using the R package *lme4*.

3 | RESULTS

We included 47 children who had asthma at age 7 and had measurements of FeNO, blood eosinophil count, sIgE, and/or skin prick test. For the control group, we included 165 children who had attended at least one visit between 6 months and 6 ½ years, as well as follow-up visits at 7, 13, and 18 years. Children with asthma at any other ages than 7 years were excluded ($n = 123$), along with 3 children who lacked biomarker measurements required for phenotype classification. See Figure 1. Participant retention varied across follow-up visits, as illustrated in Figure S1.

The 47 children with asthma at age 7 were subcategorized based on their T2-high ($n = 26$) and T2-low ($n = 21$) phenotypes. Twenty-four had eosinophilia, and 8 had elevated FeNO. Seventeen of the children were sensitized, 5 of them being mono-sensitized and 12 polysensitized. In terms of overlap, 7 children had eosinophilia only, 5 had sensitization only, and none of the children had elevated FeNO without concurrent elevations in other biomarkers. Additionally, 6 children had both eosinophilia and sensitization, another 6 had both eosinophilia and elevated FeNO, and 6 children exhibited all three characteristics (see Figure 2).

3.1 | Characteristics of T2-high and T2-low asthma

Baseline characteristics of children in the T2-high, T2-low, and control group are summarized in Table 1. Children with T2-high compared to T2-low asthma at 7 years were more likely to develop allergic rhinitis (76.9% vs. 19.0%) and atopic dermatitis (80.8% vs. 42.8%) at some point during childhood (0–18 yrs). Furthermore, children with T2-high asthma were concurrently more often sensitized to food allergens (32.0% vs. 5.0%) and had higher mean total IgE level (90.6 vs. 22.5 IU/mL).

Persistent asthma at age 18 was more prevalent in the T2-high group, with 46.2% of these children experiencing persistent asthma compared to 9.2% in the T2-low group. Furthermore, children with T2-high asthma at age 7 years had a later asthma debut (3.6 years) compared to those with T2-low asthma (1.8 years). The duration of asthma was also longer in the T2-high group, with a median duration of 10.2 years versus 5.6 years in the T2-low group ($p = .019$) (Table 1).

Lung function assessments throughout childhood revealed that sRaw at age 3–18 yrs. was 12.5% higher in children with T2-high asthma at age 7 years compared to T2-low, suggesting significantly higher airway resistance in this group ($p = .029$) (Figure 3). Despite this, spirometry results, including FEV1, MMEF, and the FEV1/FVC index, did not show significant differences between the two groups (Table 2).

Bronchodilator response analyses at age 5–18 yrs. indicated that children with T2-high asthma were more likely to exhibit significant improvement in lung function after medication as compared to those with T2-low asthma (OR 3.37 [1.03–11.00], $p = .044$; OR 2.60 [1.17; 5.75], $p = .019$, for spirometry and sRaw, respectively).

Methacholine challenge tests further highlighted that children with T2-high asthma had greater sensitivity to methacholine, indicating higher bronchial hyperresponsiveness compared to T2-low (Figure 4) ($p = .004$). Additional lung function assessments, including exercise tests and cold air provocations, did not reveal significant differences between the T2-high and T2-low asthma groups (Table 2).

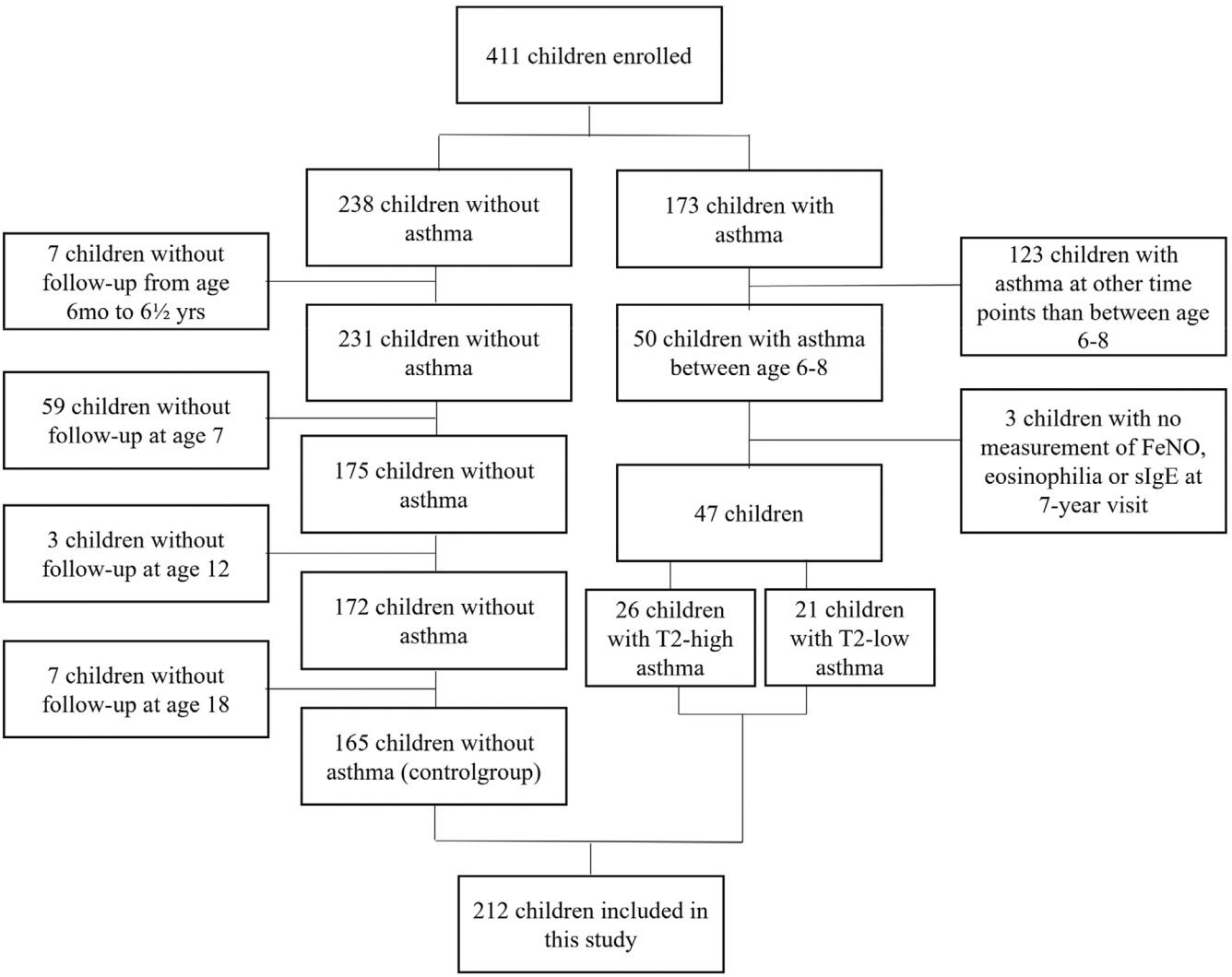

FIGURE 1 Study flowchart.

Differences in lung function results between T2-low asthma and the control group are shown in Table S1.

Exacerbations were recorded throughout the follow-up period, from age 0–18 years. Children with T2-high asthma had a median of 1 (IQR 0–4.75) exacerbation, while children with T2-low asthma had 2 (IQR 0–5), showing no significant difference in the number of exacerbations between the groups (Table 1).

4 | DISCUSSION

4.1 | Primary findings

This study of the $COPSAC_{2000}$ mother–child cohort provides insights into the pediatric T2-high asthma phenotype compared to the T2-low phenotype. Among children with asthma at age 7, T2-high asthma was more persistent, had a later age of onset, and a longer asthma duration compared to T2-low asthma. Furthermore, baseline sRaw and bronchodilator responses were increased in children with T2-high asthma, suggesting a more severe phenotype. Our findings in this study underscore the chronicity and potentially greater disease burden associated with the T2-high phenotype.

4.2 | Strengths and limitations

This study is based on data from the $COPSAC_{2000}$ cohort, thus benefiting from a robust asthma diagnosis algorithm. All children were seen at the COPSAC clinic at scheduled and acute visits for any asthma-related symptoms. The rigid algorithm in diagnosing asthma and frequent visits at COPSAC at least every 6 months enabled us to have accurate data on age of onset and remission. Further, lung function was assessed neonatally and at every visit from age 3 years. Therefore, we were able to evaluate trajectories in lung function through the entire follow-up period. This study is, to our knowledge, the first study to investigate trajectories of T2-high asthma in a pediatric population using longitudinal data.

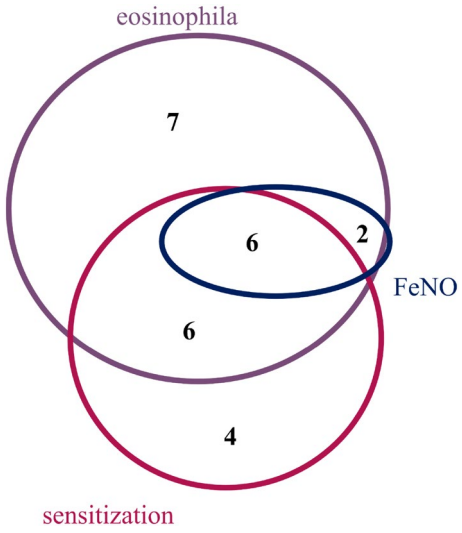

FIGURE 2 Overlap of Type 2-high asthma biomarkers (eosinophilia, FeNO, and sensitization) in children with asthma at age 7. This Venn diagram illustrates the distribution and overlap of biomarkers among the 47 children with asthma. Twenty-four children had eosinophilia, 17 were sensitized, and 8 had elevated FeNO. Of these 7 children had eosinophilia only. Five children were sensitized only. Six children had both eosinophilia and sensitization. Six children had both eosinophilia and elevated FeNO. Six children exhibited all three characteristics (eosinophilia, FeNO, and sensitization). No children had elevated FeNO without concurrent elevations in other biomarkers.

A potential limitation of this study is related to the cohort size, which may reduce the statistical power necessary for analyzing certain outcomes. Furthermore, the composition of the $COPSAC_{2000}$ cohort consisting of children at high risk of asthma with high levels of eosinophils and displaying limited ethnic diversity, may affect the applicability of our findings across broader populations.

4.3 | Interpretation

We identified a later age of onset for children with T2-high asthma at age 7 years compared to T2-low asthma and a significantly more persistent type of asthma, resulting in a longer disease duration. These findings highlight important clinical features of the T2-high asthma phenotype. Furthermore, children with T2-high asthma exhibited higher specific airway resistance and greater bronchial hyperresponsiveness to methacholine, as well as a significantly more reversible lung function throughout childhood, indicating more pronounced airway obstruction and reactivity. Despite these differences, spirometry results, including FEV1, MMEF, and the FEV1/FVC index, did not significantly differ between the T2-high and T2-low groups. Additional lung function tests, such as exercise and cold air provocations, did not reveal significant differences between the two groups.

Studies on T2-high asthma consistently highlight its persistent characteristics across different populations. The multicenter clinical All Age Asthma Cohort (ALLIANCE),[4] encompassing 1125 participants with T2-high asthma across all age groups, found that adults with T2-high asthma often had their initial asthma episodes in childhood, emphasizing the early-life origins and chronic nature of this phenotype. Similarly, the Dunedin cohort, a population-based study of 1037 children followed from birth to age 26, found that sensitization to house dust mites and AHR were strong predictors of persistent wheezing and asthma relapse into adulthood.[26] These results underscore how early-life sensitization and airway inflammation can shape long-term asthma trajectories, aligning with our findings that T2-high asthma is characterized by greater bronchial sensitivity and a prolonged disease course.

A separate study involving a large cohort of 4089 participants identified four distinct asthma trajectories from infancy to adulthood, with the persistent asthma trajectory showing elevated markers of type 2 inflammation.[27] Additionally, in another cohort, including 1925 adults, T2-high asthma was associated with a later onset and a longer duration than T2-low asthma, further underlining the severe and long-term impact of the T2-high asthma phenotype.[5]

Furthermore, our findings align with those of the German Multicentre Allergy Study (MAS90), which followed 1314 children from birth to 13 years of age.[28] The MAS90 study demonstrated that early sensitization to perennial allergens and concurrent high exposure to these allergens were associated with a loss of lung function and the development of chronic asthma by school age. Children with early perennial allergen sensitization exhibited greater AHR and lung function impairment, much like the T2-high asthma phenotype identified in our cohort. Similarly, the Tucson Children's Respiratory Study identified AHR and early sensitization to locally prevalent allergens as significant, independent predictors of chronic asthma by early adulthood.[29] This aligns with our observation that T2-high asthma is characterized by prolonged airway inflammation, AHR, and a more chronic disease trajectory.

Another study investigated remission and persistence of asthma from ages 7 to 19 years[30] and found that remission of asthma was inversely related to sensitization to furred animals and asthma severity at baseline in a cohort consisting of 3430 children. These factors are closely related to the T2-high asthma phenotype observed in our study, where persistent asthma was more common in children with higher asthma severity and sensitization. The findings of both studies emphasize the significance of sensitization and asthma severity in determining long-term asthma outcomes, highlighting the persistent nature of T2-high asthma compared to T2-low asthma.

While the specific mechanisms behind the later onset, longer duration, and more persistent nature of T2-high compared to T2-low asthma require further investigation, it could be linked to a sustained Th2-mediated immune response. This prolonged activation of the immune system, typical of T2-high asthma, may contribute to its severity making the disease more resistant to remission. Such a persistent immune response could be intertwined with airway

TABLE 1 Baseline characteristics of children at 7 years of age with T2-high versus T2-low asthma, including the control group.

Variables	T2-high ($n=26$)	T2-low ($n=21$)	p-Value, T2 high vs. T2 low	Control group ($n=165$)	Missing (high/low/control)
Biometrics					
Males, *n* (%)	14 (54)	10 (48)	.77	76 (46)	0/0/0
BMI at 7 years, Z-scores (SD)	0.35 (1.15)	0.60 (0.95)	.41	0.20 (0.87)	1/0/2
Predisposition					
Maternal asthma, *n* (%)	26 (100.0)	21 (100.0)	1.00	165 (100.0)	0/0/0
Paternal asthma, *n* (%)	9 (36.0)	5 (25.0)	.53	31 (18.9)	1/1/1
Other atopic diseases					
Allergic rhinitis, ever, *n* (%)	20 (76.9)	4 (19.0)	**1.11e-04**	53 (32.1)	0/0/0
Atopic dermatitis, ever, *n* (%)	21 (80.8)	9 (42.8)	**.01**	82 (49.7)	0/0/0
T2 biomarkers, 7 years					
Blood eosinophils, max value at 6- or 7-visit, mean (SD), 10^{-9}/L	0.66 (0.25)	0.31 (0.12)	**4.92e-07**	0.45 (0.37)	1/1/15
Blood eosinophils, mean value at 6- and 7 years visit, mean (SD), 10^{-9}/L	0.57 (0.23)	0.26 (0.11)	**8.60e-07**	0.39 (0.30)	1/1/15
FeNO, max value at 6- or 7 years visit, mean (SD), ppb	16.14 (10.95)	8.04 (3.42)	**.001**	9.88 (7.77)	0/0/0
Specific IgE to aeroallergens ≥0.35 IU/mL, *n* (%)	13 (50)	0 (0)	**3.34e-05**	30 (20.7)	3/1/20
Positive SPT, aeroallergens, *n* (%)	12 (46)	0 (0)	**9.64e-05**	15 (10.8)	3/1/26
Other biomarkers, 7 years					
Specific IgE to food allergens ≥0.35 IU/mL, *n* (%)	8 (31)	1 (5)	**.02**	25 (17.2)	1/1/20
Positive SPT, food, *n* (%)	2 (8.7)	0 (0)	.49	3 (2.2)	3/2/27
Total IgE, mean (95% CI), IU/mL	87 (45–170)	20 (10–40)	**.003**	43.55 (33.83–52.94)	3/1/20
Asthma outcomes					
Age of asthma onset, years, median (SD)	3.6 (3.45)	1.78 (1.10)	**.008**		
Asthma duration, years, median (SD)	10.23 (6.18)	5.62 (2.65)	**.019**		
Asthma persistency, %	46.2	9.2	**.013**		
Number of exacerbations requiring OCS and/or hospital admission, median (IQR)	1 (0–4.75)	2 (0–6)	.87		

Note: Bold values indicate $p<0.05$.

Abbreviations: BMI, Body mass index; FeNO, Fractional Exhaled Nitric Oxide; IgE, Immunoglobulin E; SPT, Skin prick test.

remodeling processes, where ongoing inflammation leads to structural changes in the airways,[3] further complicating the asthma phenotype. The sustained release of Th2 cytokines, including IL-4, IL-5, and IL-13, plays an important role in this process, promoting eosinophilic inflammation and contributing to the characteristic features of T2-high asthma.[3] This is consistent with evidence showing that early-life exposure to allergens, such as dust mites and pet dander, may exacerbate the immune dysregulation characteristic of T2-high asthma, amplifying eosinophilic airway inflammation and increasing the risk of persistent disease.[31] These mechanisms highlight the complex interplay between the immune system's response and airway remodeling in T2-high asthma, providing a plausible explanation for its later onset, longer duration, and persistent nature compared to T2-low asthma, indicating that eosinophilic airway inflammation is worse that non-eosinophilic airway inflammation. This understanding aligns with existing literature, emphasizing the need for targeted therapeutic strategies that address the underlying T2 inflammation and airway remodeling to effectively manage T2-high asthma.[32]

Our study identified T2-high asthma as manifesting a more severe phenotype compared to T2-low asthma, characterized by increased airway resistance as illustrated in Figure 3, which shows consistently higher sRaw Z-scores in children with T2-high asthma compared to T2-low asthma and controls across childhood. It has been demonstrated that sRaw is a reliable measurement for diagnosing asthma,

offering advantages over FEV1 in early and precise assessment of lung function.[22,33] Notably, sRaw measurements can be initiated from as young as 2 years of age, enabling earlier detection and management of asthma compared to spirometry, which is typically not used before age 5.[34] While our study did not observe a statistically significantly lower FEV1 among children with T2-high asthma, the trend towards reduced FEV1 in this group suggests the need for further investigation with larger cohorts. In comparison, the study of the NHOM cohort demonstrated significantly lower FEV1% predicted values in adults with T2-high asthma compared to those with T2-low asthma.[5] Another study found high blood eosinophil count to be a risk factor of declining FEV1% predicted.[35] Similarly, the study of the Dunedin cohort identified sensitization to house dust mites as a strong predictor of persistent asthma and lower FEV1/FVC ratios.[26] These findings emphasize the role of early allergen sensitization and eosinophilic inflammation in shaping long-term pulmonary function outcomes, aligning with our observation of increased airway resistance and eosinophilia in children with T2-high asthma.

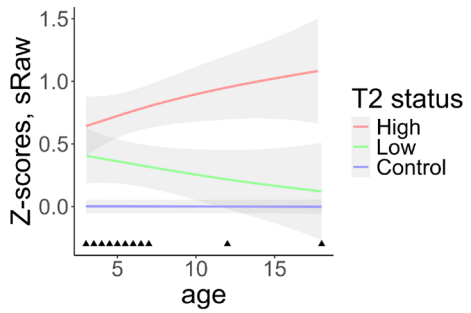

FIGURE 3 Z-scores for log-transformed specific airway resistance (sRaw) across age in T2-high versus T2-low asthma. The X-axis represents age in years, and the Y-axis shows Z-scores of log-transformed specific airway resistance (sRaw). Z-scores were normalized to the control group's mean for comparison across age. Colors represent asthma phenotypes: Red for T2-high ($n = 26$), green for T2-low ($n = 21$), and blue for the control group ($n = 165$). Triangles mark the visits when lung function was measured. Analysis using a linear mixed model revealed a significant difference in sRaw between the T2-high and T2 low asthma groups, estimate 0.53 [0.06; 1.01], $p = .029$.

Our findings from the methacholine challenge test shows heightened AHR in children with T2-high asthma, demonstrated by a greater sensitivity to methacholine. This is illustrated in Figure 4, showing that children with T2-high asthma had consistently lower Z-scores for PD15/PD20 compared to T2-low asthma and controls across childhood, indicating greater airway hyperresponsiveness. This is likely attributed to the eosinophilic inflammation and mast cell activation typical of T2-high asthma, which leads to the release of bronchoconstrictive substances enhancing methacholine sensitivity.[36]

Furthermore, the Tucson study demonstrated that early-life wheezing phenotypes, particularly persistent wheezing, strongly predict long-term asthma persistence and severity. This aligns with our findings, where T2-high asthma was associated with increased airway resistance, bronchial sensitivity, and greater asthma persistence into adolescence. These results emphasize the importance of addressing T2 inflammation early in life to mitigate the long-term impact of airway remodeling and chronic asthma.

Together, these findings underscore that T2-high asthma represents a severe, persistent phenotype driven by eosinophilic inflammation and BHR. Early identification and intervention targeting T2 inflammation could play a critical role in altering the

TABLE 2 Longitudinal lung function outcomes measured from ages 0–18 years.

Measures	Estimate [95% CI] T2 high vs. T2 low	*p*-Value
Specific airway resistance (sRaw)[a], 3–18 years	0.53 [0.06; 1.01]	**.029**
Reversibility testing, sRaw[a], 3–18 years	OR 2.60 [1.17; 5.75]	**.019**
Forced expiratory volume in 1 second (FEV1)[a], 0–18 years	−0.24 [−0.58; 0.09]	.16
Reversibility testing, FEV1[a], 5–18 years	OR 3.37 [1.03–11.00]	**.044**
FEV1/FVC-index[a], 7–18 years	0.03 [−0.06; 0.01]	.12
The mid-maximum expiratory flow (MMEF)[a], 0–18 years	−0.19 [−0.62; 0.24]	.37
Methacholine challenge test[a], 0–18 years	−0.70 [−1.18; −0.23]	**.004**
Bronchial response to exercise test, 7 years	W = 191	.54
Bronchial response to cold air, 6½ years	W = 141	.61

Note: Bold values indicate $p < 0.05$.

Abbreviation: W, Wilcoxon test statistic.

[a]Linear mixed models applied.

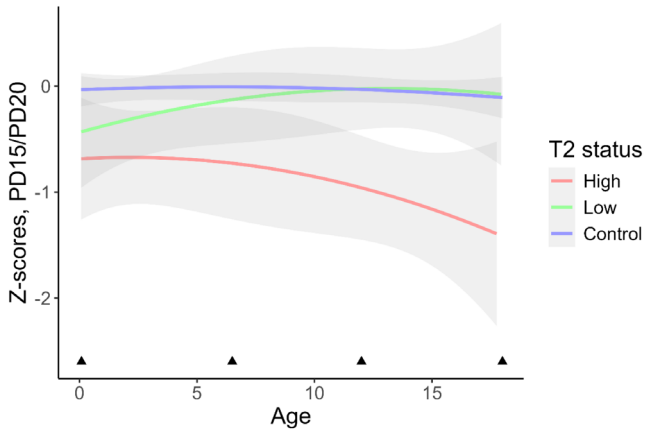

FIGURE 4 Z-scores for log-transformed methacholine sensitivity (PD15/PD20) across age in T2-high versus T2-low asthma. The X-axis represents age in years, while the Y-axis shows the Z-scores of log-transformed methacholine sensitivity (PD15/PD20), normalized to the control group's mean. Colors indicate T2 status: Red for T2-high, green for T2-low, and blue for controls. Black triangles mark the visit when methacholine sensitivity was measured (1 month, 6.5 years, 12 years, and 18 years). Linear mixed model analysis showed significantly increased methacholine sensitivity in T2-high asthma, highlighting enhanced airway hyperresponsiveness in this group (estimate −0.70 [−1.18; −0.23], $p = .004$).

trajectory of asthma, reducing morbidity, and improving long-term outcomes.

Additionally, a dysfunctional airway epithelium can produce higher levels of cytokines that amplify the inflammatory cascade and sensitize the airway smooth muscle to methacholine.[3]

While ICS have long been the cornerstone of asthma management, the emergence of biologic therapies has broadened the therapeutic landscape, especially for severe eosinophilic and T2-high asthma. In this COPSAC cohort study, all children with asthma were uniformly treated with ICS, which limited our capacity to examine differences in corticosteroid responsiveness or the effect of varying ICS dosages in T2-high and T2-low asthma. However, we observed more positive responses to bronchodilators among children with T2-high asthma, which persisted irrespective of the consistent ICS regimen, underscoring the inherently severe nature of the T2-high asthma phenotype. In line with this, a study involving 508 adults with asthma, a considerable fraction of those with marked eosinophilic inflammation continued to experience uncontrolled asthma symptoms despite receiving high doses of ICS, suggesting corticosteroid resistance or insufficiency in fully addressing the inflammatory processes associated with T2-high asthma.[37]

We observed a significant difference in methacholine sensitivity between T2-high and T2-low asthma; however, additional bronchial provocation tests, such as exercise and cold air provocations, did not show significant lung function variations. These so-called indirect tests, more suited for monitoring asthma, may have limited use in distinguishing asthma phenotypes in well-controlled cohorts where children receive regular ICS treatment and biannual outpatient visits.[38] Our study found no difference in the number of exacerbations up to age 18, likely due to most children being well-controlled in their asthma. Previous studies have demonstrated that biologics targeting T2 inflammation significantly reduce the number of exacerbations in asthma, particularly in adults,[39,40] suggesting that inhibiting T2 inflammation is effective in decreasing exacerbations. The majority of these studies focus on adults, implying that the differences in exacerbations between T2-high and T2-low asthma may become more apparent later in life. Further research is needed to determine if these distinctions emerge beyond childhood.

4.4 | Biomarkers

In defining T2-high and T2-low asthma, we selected FeNO, sensitization to aeroallergens, and blood eosinophil count as primary biomarkers. This approach aligns with prior studies demonstrating that FeNO, eosinophil count, and IgE are reliable indicators for characterizing T2-high asthma in children, particularly within the COPSAC cohort.[12,41]

Furthermore, our findings indicated that FeNO showed limited diagnostic value on its own, as none of the children had elevated FeNO without concurrent elevations in other biomarkers. This aligns with previous COPSAC studies showing that FeNO is elevated only in sensitized children with asthma,[42] raising speculation about its standalone diagnostic value and emphasizing the need to combine FeNO with sensitization status for meaningful asthma phenotyping.

4.5 | Clinical implications

Given the persistent nature and increased airway resistance of T2-high asthma, this phenotype might necessitate a more aggressive treatment strategy from a young age, including the early use of inhaled corticosteroids and possibly adjunctive therapies for those showing inadequate response. The pronounced bronchodilator responsiveness in T2-high asthma also suggests a potential benefit from regular treatment with bronchodilators.

5 | CONCLUSION

This longitudinal study of clinical features and lung function measures from birth to adulthood provides insights into the T2-high and T2-low asthma phenotypes, emphasizing a later disease onset, longer persistence, more bronchial hyperresponsiveness, higher airway resistance, and a more bronchial reversibility of the T2-high compared to the T2-low phenotype. These findings underscore a need for personalized management strategies for different asthma phenotypes based on T2-high and T2-low characteristics.

AUTHOR CONTRIBUTIONS

Frederikke R. Skov: Conceptualization (supporting); formal analysis (lead); investigation (lead); methodology (lead); project administration (lead); validation (lead); visualization (lead); writing – original draft (lead); writing – review and editing (lead). **Tamo Sultan:** Writing – original draft (supporting); writing – review and editing (supporting). **Kasper Fischer-Rasmussen:** Formal analysis (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Bo L. Chawes:** Writing – original draft (supporting); writing – review and editing (supporting). **Jakob Stokholm:** Writing – original draft (supporting); writing – review and editing (supporting). **Nilo Vahman:** Writing – original draft (supporting); writing – review and editing (supporting). **Klaus Bønnelykke:** Writing – original draft (supporting); writing – review and editing (supporting). **Ann-Marie M. Schoos:** Conceptualization (lead); formal analysis (supporting); investigation (supporting); methodology (supporting); project administration (supporting); supervision (lead); visualization (supporting); writing – original draft (supporting); writing – review and editing (supporting).

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CONFLICT OF INTEREST STATEMENT

AMS: Advisory board for ALK-Abello and paid speaker for ThermoFisher Scientific. All other authors declare no potential, perceived, or real conflict of interest regarding the content of this manuscript. The funding agencies did not have any role in design and conduct of the study; collection, management, and interpretation of the data; or preparation, review, or approval of the manuscript. No pharmaceutical company was involved in the study.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/pai.70032.

GOVERNANCE

We are aware of and comply with recognized codes of good research practice, including the Danish Code of Conduct for Research Integrity. We comply with national and international rules on the safety and rights of patients and healthy subjects, including Good Clinical Practice (GCP) as defined in the EU's Directive on Good Clinical Practice, the International Conference on Harmonization's (ICH) good clinical practice guidelines, and the Helsinki Declaration. Privacy is important to us which is why we follow national and international legislation on General Data Protection Regulation (GDPR), the Danish Act on Processing of Personal Data, and the practice of the Danish Data Inspectorate.

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SUPPORTING INFORMATION

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

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An explainable machine learning approach on early predictors of persistent childhood asthma and allergic rhinitis

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Governance: We are aware of and comply with recognized codes of good research practice, including the Danish Code of Conduct for Research Integrity. We comply with national and international rules on the safety and rights of patients and healthy subjects, including Good Clinical Practice (GCP) as defined in the EU's Directive on Good Clinical Practice, the International Conference on Harmonisation's (ICH) good clinical practice guidelines and the Helsinki Declaration. Privacy is important to us which is why we follow national and international legislation on General Data Protection Regulation (GDPR), the Danish Act on Processing of Personal Data and the practice of the Danish Data Inspectorate.

Abbreviations:

AR = Allergic Rhinitis

AUC = Area Under the Curve

$COPSAC_{2000}$ = Copenhagen Prospective Studies on Asthma in Childhood 2000

DGGE = Denaturing Gradient Gel Electrophoresis

ENet = ElasticNet

ERJ = European Respiratory Journal

FeNO = Fractional Exhaled Nitric Oxide

ICS = Inhaled Corticosteriods

MCC = Matthew's Correlation Coefficient

MMEF = Maximum Mid-Expiratory Flow

RF = Random Forest

SHAP = Shapley Additive Explanations

sIgE = Specific Immunoglobulin E

SPT = Skin prick test

Abstract

Background: Asthma and allergic rhinitis (AR) are common chronic childhood diseases influenced by an interplay between genetic, environmental, and immunological factors, but traditional statistical methods often fail to capture such non-linearities and interactions. This study aimed to identify early-life determinants of asthma and AR persistence in childhood using explainable machine learning models.

Methods: Data from 411 children in the $COPSAC_{2000}$ cohort were analyzed. Using 113 early-life features across genetic, environmental, immunological, and clinical domains, we trained four machine learning models (Random Forest, LightGBM, ElasticNet, and Deep Neural Network) on four classification tasks:

- persistent asthma (diagnosis after age 17 in children with prior childhood asthma) vs controls,
- persistent asthma vs asthma with remission (no asthma diagnosis after age 17 despite a documented childhood asthma diagnosis),
- persistent AR (diagnosis at age 7 and 18) vs controls, and
- teen-onset AR (AR diagnosis at age 13 and/or 18 without AR diagnosis at age 7) vs controls.

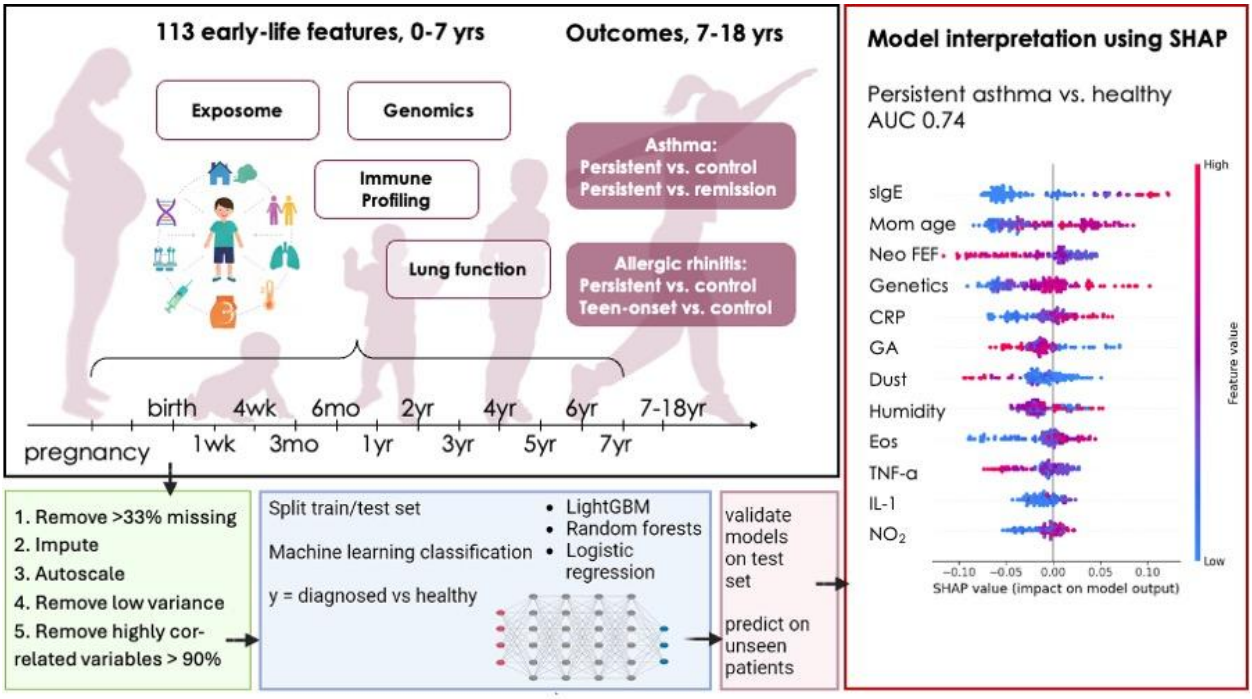
Feature importance and model interpretability were assessed using SHapley Additive exPlanations (SHAP).

Results: Predictive performance varied across models and outcomes, with only one classification task (persistent asthma vs controls) achieving an AUC above 0.7 (Random Forest and

LightGBM). No model performed adequately for AR outcomes (AUC ≤0.6), and SHAP revealed no consistent evidence of meaningful interactions among features.

Conclusion: Even with a uniquely detailed dataset and advanced machine learning methods, early-life predictors alone were insufficient for predicting asthma and AR trajectories into adolescence. These findings suggest that remission and persistence may depend on later-life exposures or stochastic disease processes, and highlight the current limitations of machine learning-based prediction in early childhood research.

Graphical abstract



Key message

Using multiple machine learning approaches applied to this early-life dataset, we evaluated whether long-term trajectories of asthma and allergic rhinitis could be predicted in early childhood. While some early-life factors were associated with persistent asthma, we were unable to identify robust combinations or interactions of early clinical, immunological, genetic, and environmental factors that reliably predicted asthma persistence or remission, or allergic rhinitis outcomes. This limited prognostic performance may reflect several factors, including constraints in cohort size, limited information content in early-life data, or the possibility that clinically relevant patterns and interactions emerge later in childhood. Clinically, the findings highlight the current uncertainty surrounding early prognostic stratification in pediatric asthma and allergic rhinitis.

Keywords

1) Asthma; 2) asthma: clinical aspects; 3) asthma: early intervention; 4) asthma: risk factors; 5) rhinitis: 6) allergic; rhinitis; 7) rhinitis: risk factors

Introduction

Asthma and allergic rhinitis (AR) are among the most common chronic diseases affecting children and adolescents worldwide, with substantial implications for quality of life and long-term health (1). Early-life risk factors include parental atopic disease (2), genetic variants (3–9), prenatal smoking (10,11), air pollution (12,13), elevated cytokines (2,3), airway infections (4–6), and allergic sensitization (8,9), all of which are well established. However, traditional statistical models often assume linear and additive effects, limiting their ability to reflect the complexity of atopic disease development.

The trajectories of asthma and AR are likely shaped by non-linear relationships and interactions, where the effect of one factor may depend on the presence of another - for example, genetic predisposition may only increase disease risk under certain environmental conditions. Moreover, asthma and AR persistence may involve an element of random variation, making remission or progression only partially predictable from early-life data.

Machine learning offers a flexible framework to model such complexity by capturing both non-linear patterns and interactions without strong prior assumptions. In this study, we applied explainable machine learning models to rich early-life data from the Copenhagen Prospective Studies on Asthma in Childhood ($COPSAC_{2000}$) aiming to identify predictors of asthma and AR persistence into adolescence.

Methods

Study population

The $COPSAC_{2000}$ cohort is a prospective, single-center study comprising 411 children born between 1998-2001 to mothers with a history of asthma. The children were seen by COPSAC physicians every 6 months between ages 0-7 years, and at 13 and 18 years. Study design and rationale has previously been described in detail (14).

Ethics

The Copenhagen Ethics Committee (KF 01-289/96) and The Danish Data Protection Agency (2015-41-3696) approved the study. Oral and written informed consent was obtained from both parents at enrolment.

Asthma diagnosis

Asthma was diagnosed and managed solely by COPSAC physicians. The diagnosis was based on four comprehensive criteria: a) recurrent episodes of troublesome lung symptoms, defined as at least five episodes lasting three consecutive days within six months, or daily symptoms for four consecutive weeks, b) typical asthma symptoms, such as prolonged nocturnal cough, exercise-induced symptoms, and symptoms causing nighttime awakenings, c) effective response to intermittent use of inhaled β2-agonists, and d) a positive response followed by a relapse after a three-month course of inhaled corticosteroids (ICS). A relapse was identified as either two episodes of symptoms lasting three days within three months, or symptoms occurring more than twice per week for two consecutive weeks. Severe acute symptoms or exacerbations requiring hospitalization or oral corticosteroids also led to an asthma diagnosis, initiating a six-month

course of ICS treatment. Asthma remission was considered when there were no symptoms or need for asthma treatment for one year.

Following the initial diagnosis, asthma was retrospectively validated objectively using the European Respiratory Society (ERS) clinical practice guideline criteria from age 6 years, that take into account the results of lung function tests (15). Persistent asthma was defined as having symptoms after age 17 with a previous diagnosis; those with onset after age 17 without a prior diagnosis were excluded. Asthma with remission was defined as having a diagnosis established before age 17, but no diagnosis at or beyond this age. The control group was defined as children who attended at least one visit between the ages of 0 and 6.5 years, as well as the 7-, 13-, and 18-year visits, and who never received a diagnosis of asthma.

Allergic rhinitis

AR was assessed at ages 7, 13, and 18 years and the diagnosis was based on parental interviews reporting symptoms such as troublesome sneezing, nasal congestion or rhinorrhea, and/or ocular symptoms (watery, itchy, swollen, or red eyes), which significantly impaired the child's well-being in the absence of concurrent cold or flu. To ensure objective validation, the diagnosis also required either positive skin-prick test (SPT) or elevated specific IgE (sIgE) levels to relevant aeroallergens. If there were no signs of sensitization to relevant allergens, the child was diagnosed as non-AR. Sensitization to common aeroallergens was determined using SPT (ALK-Abelló, Copenhagen, Denmark) and sIgE measurements (ImmunoCAP; ThermoFisher Scientific, Uppsala, Sweden). A positive test was defined as a mean wheel diameter of 3 mm larger than the negative control and/or sIgE levels ≥0.35 kU/L. Children were categorized based on their AR status at different ages. Teen-onset AR was defined as the absence of AR at age 7 years followed

by its presence at ages 13 and/or 18 years. Persistent AR was characterized by the presence of AR at both ages 7 and 18 years.

The control group was defined as children who attended at least one visit between the ages of 0 and 6.5 years, as well as the 7-, 13-, and 18-year visits, and who never received a diagnosis of AR or non-AR.

Early life exposures

We selected 113 variables collected during early life (0-7 years) to assess their influence on the development and remission of asthma and AR. These variables encompassed a broad range of categories, including atopic diseases, biometric measurements, birth-related variables, exposome factors, genetics, immune profiles, lung function, maternal and paternal predispositions, pregnancy-related exposures, and social circumstances. For the persistent asthma versus control outcome, we excluded lung function measurements as exposure variables (n=11), as they were part of the criteria used to validate asthma cases. In the asthma with remission vs persistent asthma analysis, we kept the lung function measurements as they were not used to differentiate asthma with remission from persistent asthma.

For AR, we excluded 14 features, most importantly, we removed the sIgE measurements as exposure variables as these were part of the definition of AR.

All features are listed in **Table E1**.

Machine learning

For asthma outcomes, comparisons were made between persistent asthma vs asthma with remission and persistent asthma vs controls, to explore different phenotypes. For AR outcomes, comparisons were made between persistent AR vs controls and between children with teen-onset AR and controls. We trained and validated a total of 16 machine learning models (i.e., 4 algorithms for the 4 outcomes - 2 for AR and 2 for asthma) to classify outcomes for asthma and AR, and evaluated the models on a test set of 40% of unseen data drawn randomly from the cohort.

Feature selection

We performed pre-feature selection using the following criteria for exclusion: 1) low variance (features whose scaled values fell below the 10th percentile of variance), 2) high correlation (removing features with more than 90% correlation by Spearman's coefficient) and/or 3) strong imbalance (9:1). This reduced the number of features to 83 entering the machine learning models. For in-training feature selection, we applied feature selection (during cross-validation and before testing on the test set) to avoid overfitting, based on permutation importance (16). The relative decrease in model predictive power for permuted features measured by Matthew's Correlation Coefficient (MCC) was then used to understand the importance of each feature. The elimination procedure was conducted in multiple stages. Data processing steps, including exclusion criteria for low variance, high correlation, and missingness, are detailed in the Supplementary data under Data Processing Details.

Model training

We analyzed the outcomes asthma and AR as binary classification outcomes, assigning each participant to Group 0 or Group 1. Four different machine learning methods, Random Forest (17), Elastic Net Logistic Regression (ENet) (18), Deep Neural Network (19), Light Gradient Boosting-Machine (LightGBM) (20) were used to predict the affiliation with Group 0 or 1 (per phenotype) based on the cleaned feature set. The features were matched per outcome given the feature selection procedure.

The models were implemented in Python (https://www.python.org/, v3.7.10) and trained on 60% of the data while 40% was reserved for testing. Hyper-parameter optimization was conducted using a 5-fold cross-validation hyper-parameter optimization and Bayesian optimization (21) on

the train set. The class imbalance robust statistics: Balanced Accuracy Score, Area Under the Curve (AUC), and MCC (20) were used as evaluation metrics for model performance. We interpreted an AUC of 0.5 to indicate performance equal to guessing, and an AUC of 0.6 as the threshold for "fair" predictive quality on a population level (22).

Model interpretability

To interpret the machine learning models, we used SHapley Additive exPlanations (SHAP) feature importance analysis. SHAP values quantify the contributions of individual features to the model's performance both globally and locally (23). To explore non-linear effects and potential interactions between features, we applied SHAP dependence plots as part of the model interpretation workflow. For each selected feature, we plotted the SHAP value (i.e., the feature's contribution to the prediction) against the raw feature value. This enabled visualization of both the direction and shape of the relationship, such as threshold effects or U-shaped associations. To assess potential feature interactions, we color-coded each plot by the value of a second feature, allowing us to visually inspect whether the effect of a given predictor varied depending on another variable. This method builds on the theoretical framework of SHAP, which provides local accuracy and consistency, and allows for systematic interpretation of both main effects and interactions learned by the models.

Results

Of the 176 children initially diagnosed with asthma, 163 experienced onset before age 17 years. Of these, 146 had their asthma diagnosis validated according to the ERS criteria. Subsequently, we categorized the children with asthma in two groups: asthma with remission (n=54), and persistent asthma (n=92). The control group consisted of 165 children who never developed asthma. Fifty (34%) of the children with validated asthma had already received their diagnosis before the age of 7, corresponding to the time window in which most predictor variables were collected.

For AR, we identified 90 children with teen-onset AR and 31 children with persistent AR. These groups were compared to a control group of 93 children who never developed AR or non-AR at any assessed age.

Machine learning model results

The overall performances of all the machine learning models across the different outcomes are illustrated in **Figure 1**.

Persistent asthma vs controls

The RF model demonstrated the highest predictive performance for distinguishing between children with persistent asthma and healthy controls, achieving an AUC of 0.74 (**Figure 1**). SHAP analysis was then applied to interpret the model, identifying 12 features as the most important contributors to its predictions (**Figure 2**). The sum of sIgE to aeroallergens at 6 years was the most impactful predictor, with higher levels associated with an increased likelihood of persistent asthma.

Other significant predictors for persistent asthma included higher maternal age, lower neonatal lung function (FEF 0.5), a higher genetic risk score for asthma, elevated c-reactive protein (CRP) levels at 6 months, lower gestational age, lower levels of the house dust mite allergen, *Dermatophagoides farinae* (Der f 1) in dust at 1 year, elevated eosinophil counts at 18 months, lower TNF-α levels at 6 months, and higher NO_2 exposure in the child's bedroom at 1 year.

Two features - humidity levels in the child's bedroom at 1 year and IL-6 levels at 6 months - demonstrated non-linear relationships with asthma persistence. Humidity showed that both low (blue) and high (red) levels were associated with an increased risk of persistent asthma, while moderate (purple) humidity levels were more frequently observed in children without persistent asthma, suggesting a potential "U-shaped" effect. IL-6 levels at 6 months revealed that moderate levels were more strongly linked to persistent asthma, whereas lower levels appeared less associated with disease persistence.

Persistent asthma vs asthma with remission

The LightGBM model demonstrated the highest predictive performance for distinguishing between children with persistent vs asthma with remission, achieving an AUC of 0.64 (**Figure 1**). The SHAP analysis revealed that total IgE at 4 years had the highest impact on model predictions, followed by concentrations of the dog allergen *Can f 1* (Canis familiaris allergen 1) in bed dust at age 1, mid-maximal expiratory flow (MMEF) at 7 years, airway infections from 0 to 3 years, and number of older siblings at home. Higher MMEF, frequent early-life airway infections, and having more older siblings were all associated with a lower predicted risk of persistent asthma. In contrast, the effect of total IgE at age 4 and *Can f 1* levels in early dust

samples was more complex, with both high and low values affecting the prediction variably (**Figure 3**).

Allergic Rhinitis

The ENet model was the better predictor (AUC = 0.59) distinguishing between persistent AR vs controls. However, the machine learning models were notably less effective in predicting teen-onset AR, with none of the models achieving an AUC higher than 0.49 (**Figure 1**). Given the performance of the other models, we proceeded with the ENet model for the persistent AR vs controls outcome for further analysis.

The SHAP analysis (**Figure 4**) identified the most impactful predictors of persistent AR. Father's history of allergic rhinitis was the strongest predictor, with higher values associated with persistent AR. Birth in summer was associated with a lower likelihood of persistent AR. Mother's history of allergic rhinitis, higher household income at 6 years, fewer airway infections between 0–1 year, fewer courses of antibiotics during infancy, higher sRaw at 4 years, higher eosinophil levels at 4 years, positive cold air provocation test at 6 years, lower MMEF values at 7 years, low Can f 1 dust levels in child's bedroom at 1 yr, and elevated IL-6 levels at 6 months were all associated with persistent AR. Unexpectedly, no exposure to passive smoking during pregnancy was associated with persistent AR.

Interactions

We explored potential interactions between predictors in the best-performing model, the Random Forest distinguishing persistent asthma from controls, using SHAP dependence plots. For each of the most important features identified in the SHAP summary plot (**Figure 2**), we generated an

individual dependence plot to examine whether the effect of that feature on the model's prediction was influenced by another variable. In these plots, such interactions would appear as vertical spread in SHAP values at similar feature levels, with the color of the points representing the value of a second feature. This visual pattern would indicate that the predictive contribution of a variable changes depending on another variable. However, we did not observe clear evidence of such interactions in any of the plots.

Discussion

Primary findings

Despite applying advanced machine learning models to a uniquely rich and deeply phenotyped dataset – with 113 early-life features spanning atopic diseases, biometric measurements, birth-related variables, exposome factors, genetics, immune profiles, lung function, maternal and paternal predispositions, pregnancy-related exposures, and social circumstances – we found limited and inconsistent predictive performance across asthma and AR outcomes. While only two models reached AUCs above 0.7, both for persistent asthma vs controls, all other classification tasks failed to generalize, and none performed well enough to suggest clinical utility.

This suggests that even with detailed early-life data, the remission of asthma and the development and persistence of AR into late adolescence cannot be reliably predicted from data obtained before the age of 7 years, pointing to either unmeasured exposures later in life – or an inherently unpredictable, possibly stochastic, disease course.

Strengths and limitations

The study is based on data from the $COPSAC_{2000}$ cohort, benefiting from an extensive and detailed dataset. The children were closely followed with visits every 6 months during the first seven years of life, enabling comprehensive and accurate longitudinal data collection of the early life exposome and on asthma and AR onset, progression, and remission.

Furthermore, the inclusion of 113 early-life features allowed for the evaluation of a broad range of potential predictors across domains, including genetics, environment, immunity, and lung function. However, an important limitation of using multivariate machine learning models is that they do not provide direct estimates of uncertainty for individual predictors, such as confidence intervals or p-values, limiting interpretability at the variable level.

Finally, the sample size, though large for a clinical cohort with deep phenotyping, may still be limited for high-dimensional machine learning analyses, and the inclusion of only high-risk children may reduce generalizability to broader populations.

Interpretation

The limited and inconsistent predictive performance of our machine learning models, despite the extensive phenotyping available in the COPSAC2000 cohort, challenges the notion that asthma and AR trajectories can be reliably predicted from early-life data. Although machine learning is commonly regarded as a powerful tool for capturing complex, non-linear relationships and high-dimensional interactions, our findings suggest that the persistence and remission of these diseases may instead be shaped by later-life exposures or by inherently stochastic elements in disease development.

From a methodological perspective, we employed four distinct machine learning algorithms, including both linear (ENet) and non-linear approaches (Random Forest, LightGBM, and Deep Neural Network). The ENet performed best for persistent AR vs controls, while tree-based models outperformed others in asthma-related tasks, particularly the RF model for persistent asthma vs controls. The Deep Neural Network model performed relatively poorly, despite its

theoretical flexibility, which may be attributable to overfitting due to the moderate sample size, as Deep Neural Networks generally require larger datasets to realize their potential without compromising generalizability (21).

Although tree-based models theoretically allow for uncovering complex interactions, we found no clear evidence of such effects in SHAP dependence plots. For example, no meaningful interaction between genetic predisposition and environmental exposures emerged, despite previous suggestions in the literature that genetic risk for asthma may be modulated by environmental context. Similarly, while we observed some non-linear effects, such as U-shaped associations for humidity and IL-6 levels, these patterns were isolated and did not reflect broader network-like dependencies among predictors. This absence of structured interactions suggests either that our current modeling setup is insufficiently powered to detect them, or that such interactions are less critical in determining long-term outcomes.

Furthermore, the predictive features that did emerge do not always align with established clinical expectations. Some features traditionally linked to asthma or AR, such as atopic dermatitis (24) or mode of delivery (25), were not ranked highly in any SHAP analysis of persistent disease. This could indicate that these features, although previously considered important, did not carry enough independent predictive information in this cohort to meaningfully influence the model's performance, or simply are not good predictors of disease trajectory. Conversely, elevated total IgE at 4 years and especially the sum of specific IgE to aeroallergens at 6 years were among the top predictors of persistent asthma, consistent with the role of allergic sensitization and type 2 inflammation in asthma chronicity (26–30). However, it is worth noting that we modeled sIgE as

a continuous sum, deviating from the more common use of binary thresholds or sensitization counts.

Taken together, these findings suggest that machine learning, at least as applied to early-life data alone, does not currently provide sufficient added value for long-term prognosis of asthma or AR. While the strength of machine learning lies in its capacity to screen across numerous domains and detect subtle, complex relationships, the lack of consistent, high-performing models in our study indicates that key determinants of persistence may lie beyond the scope of early-life data, potentially in later environmental exposures, epigenetic changes, or yet unidentified modifiers.

Conclusion

In this study, we used machine learning models to a detailed early-life dataset in an attempt to predict the persistence or remission of asthma and allergic rhinitis by adolescence. Despite leveraging a wide array of predictors, including genetic profiles, immune markers, environmental exposures, and lung function, the models showed limited and inconsistent predictive performance, and no evidence of meaningful interactions among features. As such, our results suggest that even advanced machine learning techniques provide little added value for long-term prediction of asthma and AR outcomes based solely on early-life data.

The inability to capture remission or persistence of asthma and AR highlights a potential role of later-life exposures or intrinsically unpredictable mechanisms in shaping disease trajectories. While the models allowed us to examine model structure and understand individual feature contributions, the absence of consistent predictors—alongside the underperformance of variables traditionally considered important—emphasizes the challenges of forecasting multifactorial diseases in a high-risk cohort. Future research should consider integrating data from later developmental stages and novel data modalities to better understand the dynamic nature of allergic and respiratory disease development and persistence.

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Figures

Figure 1

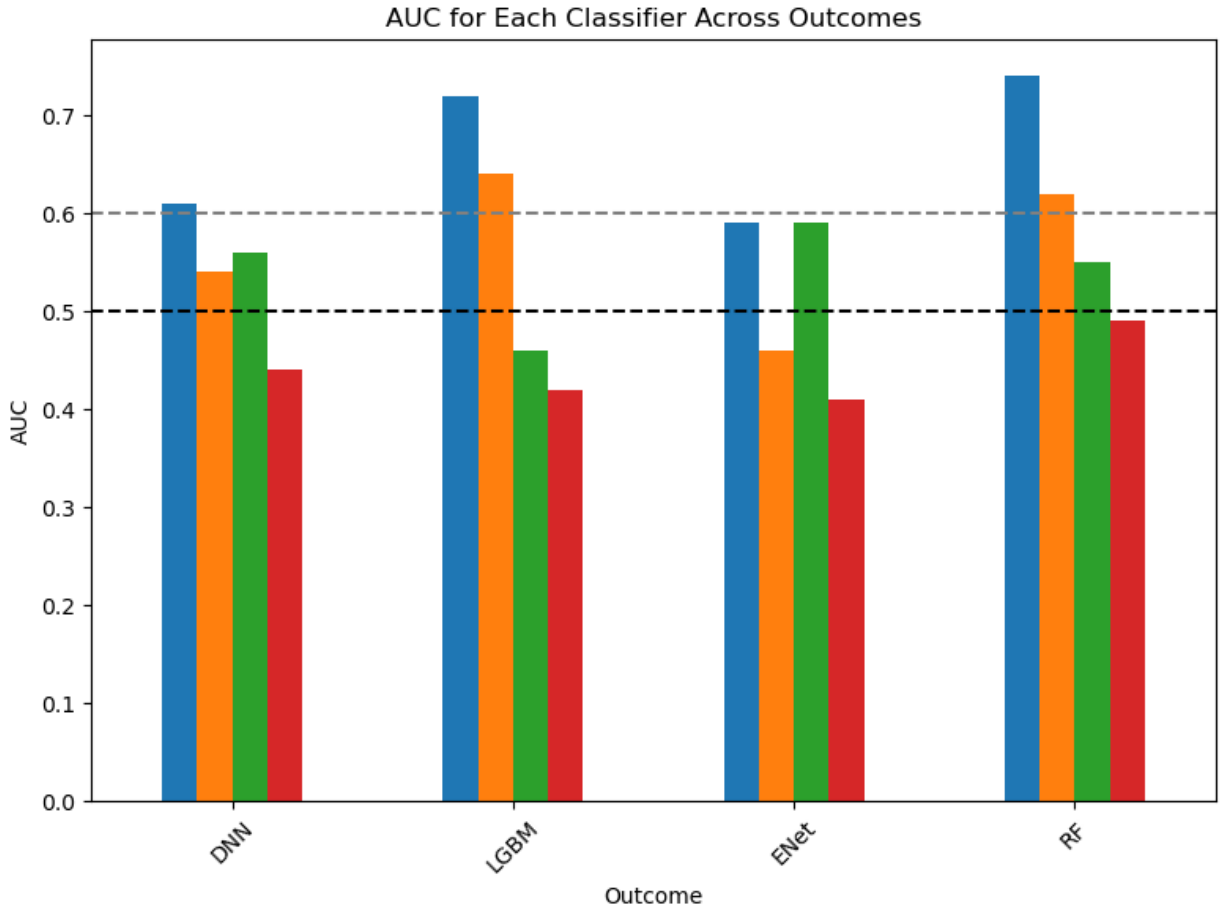


Figure 2

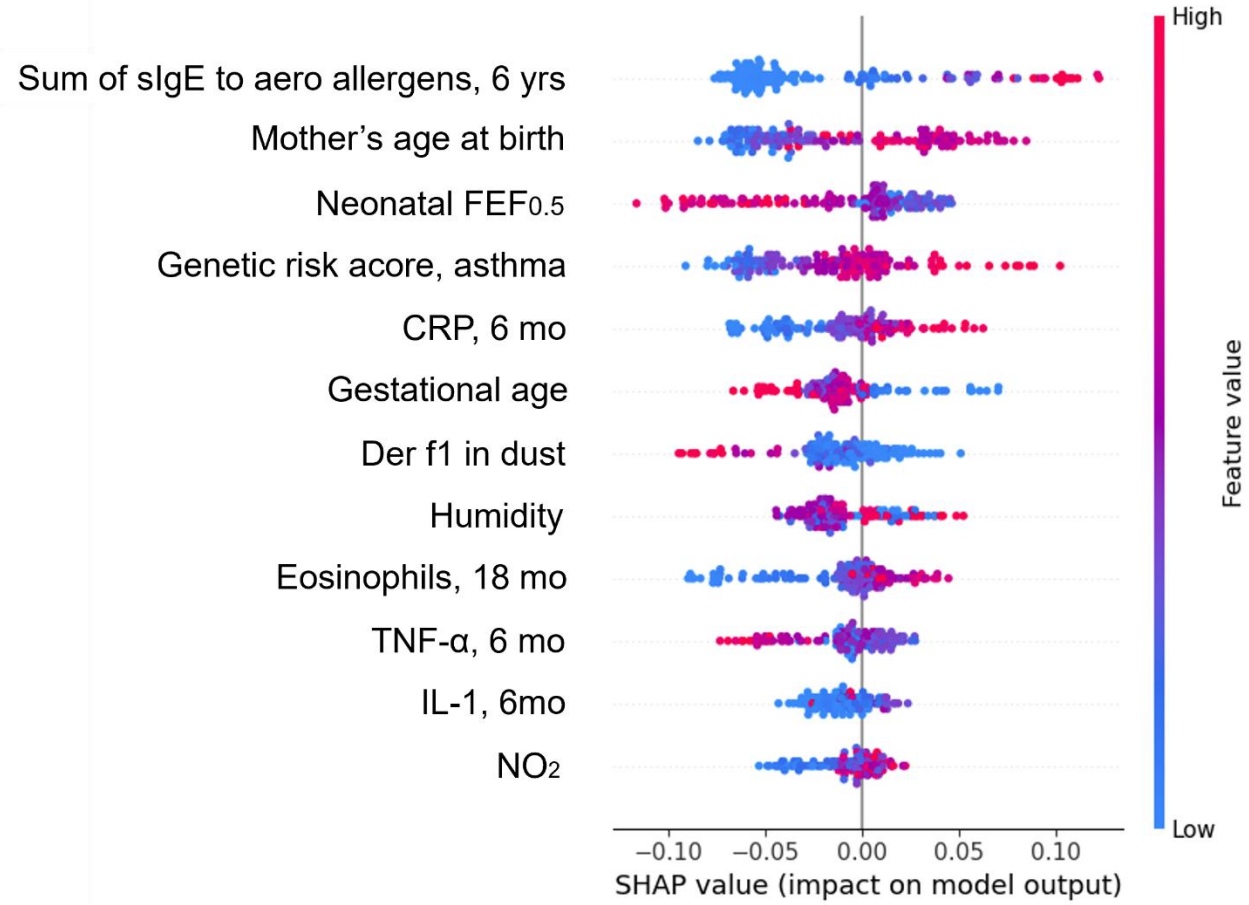

Sum of sIgE to aero allergens, 6 yrs
Mother's age at birth
Neonatal FEF0.5
Genetic risk acore, asthma
CRP, 6 mo
Gestational age
Der f1 in dust
Humidity
Eosinophils, 18 mo
TNF-α, 6 mo
IL-1, 6mo
NO2
−0.10
−0.05
0.00
0.05
0.10
SHAP value (impact on model output)
High
Feature value
Low

Figure 3

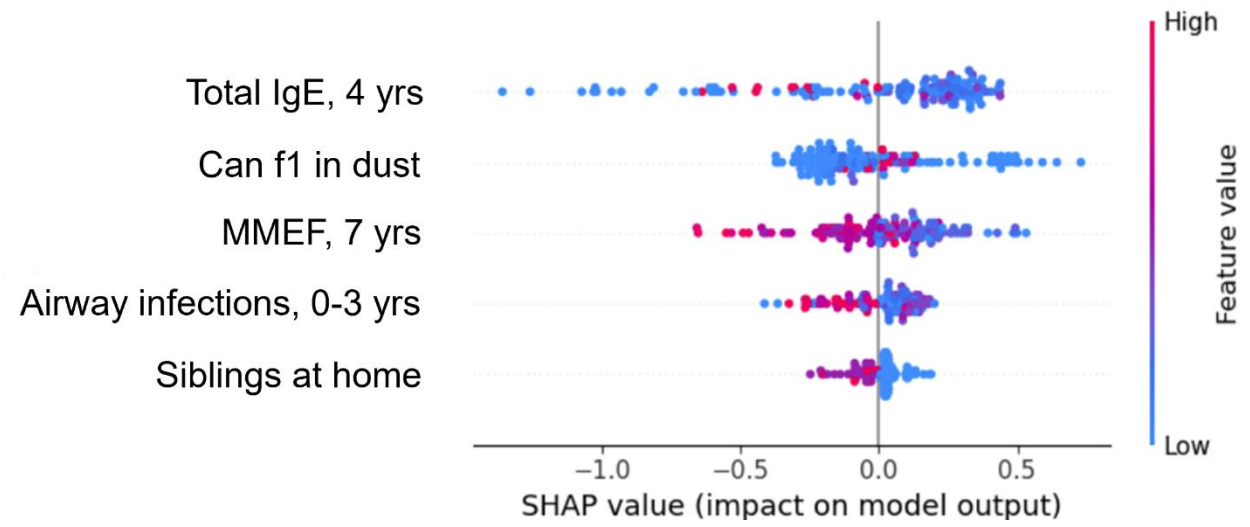
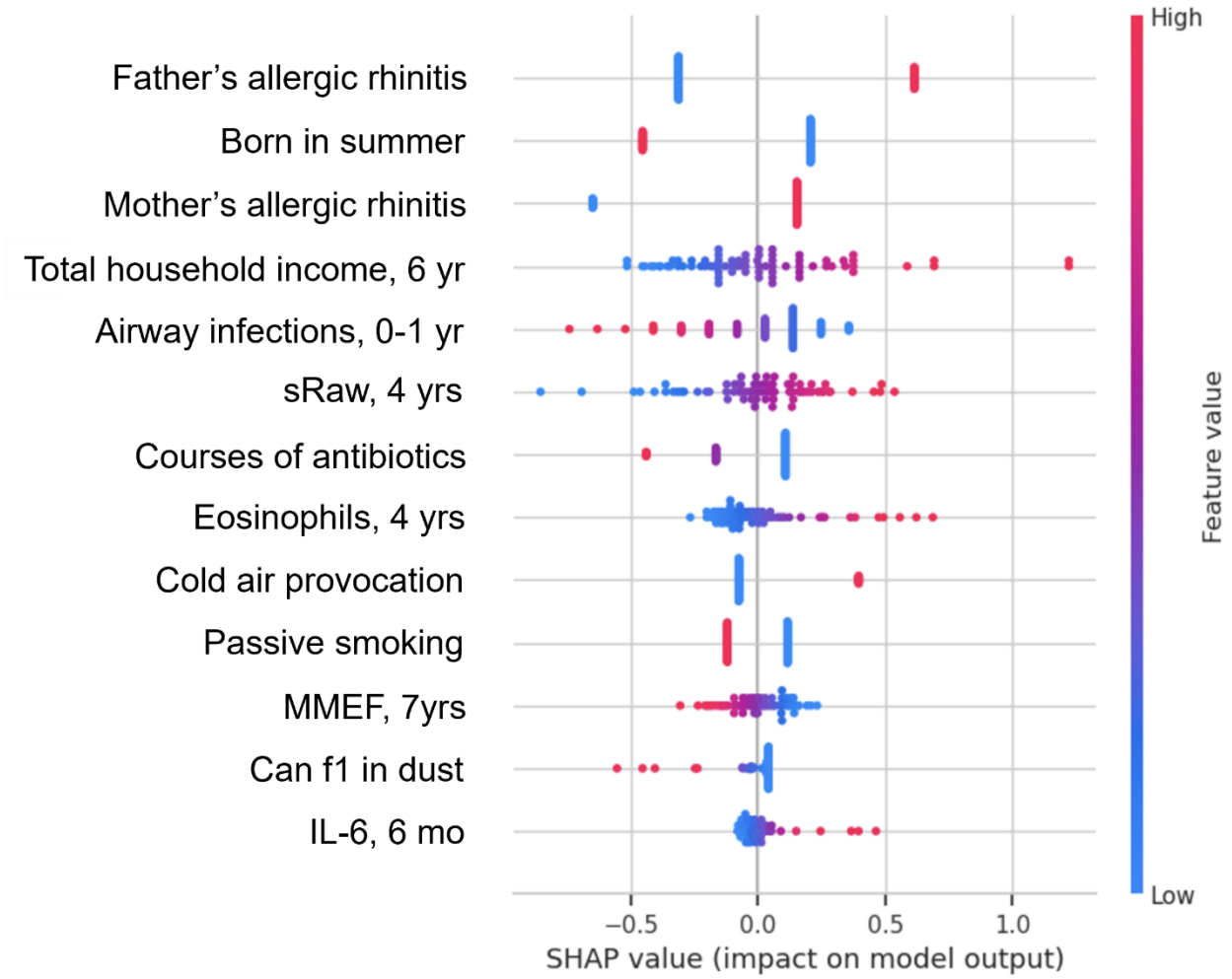

Total IgE, 4 yrs
Can f1 in dust
MMEF, 7 yrs
Airway infections, 0-3 yrs
Siblings at home
−1.0
−0.5
0.0
0.5
SHAP value (impact on model output)
High
Feature value
Low

Figure 4


Father's allergic rhinitis
Born in summer
Mother's allergic rhinitis
Total household income, 6 yr
Airway infections, 0-1 yr
sRaw, 4 yrs
Courses of antibiotics
Eosinophils, 4 yrs
Cold air provocation
Passive smoking
MMEF, 7yrs
Can f1 in dust
IL-6, 6 mo
−0.5
0.0
0.5
1.0
SHAP value (impact on model output)
High
Feature value
Low

LEGENDS

Figure 1. AUC model results per model and outcome. Each bar represents the performance of a different machine learning model for predicting asthma and AR. The black dashed horizontal line marks the AUC threshold of 0.5. The gray dashed horizontal line marks the AUC threshold of 0.6. Blue = Persistent asthma vs control group. Orange = Persistent asthma vs asthma with remission. Green = Persistent AR vs control group. Red = Teen-onset AR vs control group.

Figure 2. SHAP summary plot for the RF trained on persistent asthma vs control group. Positive SHAP values indicate a higher predicted risk of the outcome, while negative values suggest a lower risk. Furthermore, The SHAP analysis showed that the predictors with the strongest impact on the likelihood of persistent asthma are those ranked highest in the plot.

Figure 3. SHAP summary plot for the LGBM model trained on persistent vs asthma with remission. Each dot represents a Shapley value for a specific feature and instance, illustrating the feature's contribution to shifting the model output from the base value. The y-axis lists features in descending order of importance, with the most impactful features at the top. The x-axis represents the SHAP value, indicating the feature's contribution to the prediction. Positive SHAP values push the prediction higher (towards a positive outcome or 1), while negative values push it lower (towards 0). The color of the dots corresponds to the feature value, with red indicating high feature values and blue indicating low feature values. The distribution of dots reflects the variance in feature impact across different instances, indicating that some features exhibit significant influence with both positive and negative contributions depending on their values.

Figure 4. SHAP summary plot for the Elastic Net model trained on persistent AR vs control group. Positive SHAP values indicate a higher predicted risk of the outcome, while negative values suggest a lower risk. Furthermore, The SHAP analysis showed that the predictors with the strongest impact on the likelihood of persistent AR are those ranked highest in the plot.

SUPPLEMENTARY

Table E1

		AR	Asthma		Excluded during data processing
Feature	**Age of measurement (yr)**	**Both outcomes**	**Persistent vs control**	**Persistent vs asthma with remission**	
Atopic disease					
Asthma, 6-8yrs (Y/N)	7				
Atopic dermatitis, 6-8yrs (Y/N)	7				
Asthma, 0-7yrs	*				
Atopic dermatitis, 0-7yrs	*				
Biometrics					
Fat, gynoid region	6				E
Fat, android region	6				E
Fat, trunk region	6				E
Fat, TBLH	6				E
BMI, 1 yr	1				
BMI, 6 yrs	6				
Waist circumference, 6 yrs	6				E

Sex	0				
Birth-related					
Cesarean delivery (Y/N)	0				E
Apgar score at 5 minutes	0				
Gestational age (days)	0				
Alcohol intake during pregnancy (unit per week)	P				
Alcohol intake during pregnancy (Y/N)	P				E
Courses of antibiotics during pregnancy	P				
Smoking during pregnancy, days	P				
Smoking during pregnancy (Y/N)	P				E
Exposure to passive smoking during pregnancy, days	P				
Birth weight	0				
Use of paracetamol in 3rd trimester	P				
Environmental factors					
Felis domesticus allergen 1 (Fel d1) in dust	1				E
Dermatophagoides farinae allergen 1 (Der f1) in dust	1				

Dermatophagoides pteronyssinus allergen 1 (Der p1) in dust	1				
Canis familiaris allergen 1 (Can f1) in dust	1				
Born in winter	0				
Born in spring	0				
Born in summer	0				
Born in autumn	0				
Siblings at home, number	0				
Nitric dioxide	1				
Nitric oxide	1				E
Formaldehyd	1				E
Small particles	1				
Blackening	1				
Temperature in child’s bedroom	1				
Humidity	1				
Nicotine in hair	1				E
Cotinine in hair	1				
Gas stove	0-1				
Cat exposure	0-1				

Dog exposure	0-1				
Fireplace	0-1				
Dog exposure, 1st year (Y/N)	1				E
Cat exposure, 1st year (Y/N)	1				E
Airway infections, 0-3 yrs	0-3				
Airway infections, 0-1 yr	0-1				
Institution start	*				
Airway bacteria, 4 weeks	0				
EPX:creatinine ratio, 4 wks	0				E
Exclusively breastfed	*				
Partially breastfed	*				
Fish exposure	*				
Mother's age at birth	0				
Mother's education	1				
Father's education	1				
Mother's education	6				
Father's education	6				E
Total household income	1				
Total household income	6				

Predisposition					
Filaggrin mutation					
Genetic risk score, asthma					
Genetic risk score, COPD					
Genetic risk score, sensitization					
Mother's total IgE	P				
Mother's allergic rhinits	P				
Mother's max sIgE to aeroallergens	P				E
Mother's atopic dermatitis	P				
Mother's sum of sIgE to aero allergens	p				E
Mother’s food allergy					
Father's sum of sIgE to aero allergens	P				
Father's allergic rhinitis	P				
Father's atopic dermatitis	P				
Father's max sIgE to aeroallergens	P				E
Father's asthma	P				
Father's total IgE	P				E
Father’s food allergy					

Immune profile					
Sum of sIgE to food allergens, 6yrs	6				
C-reactive protein, 6mo	1				
sIgE max food allergens, 6yrs	6				E
Sum of sIgE to food allergens, 4yrs	4				E
Total IgE, 6yrs	6				E
Interleukin 1, 6mo	1				
sIgE max food allergens, 4yrs	4				E
Sum of sIgE to aero allergens, 6yrs	6				
Sum of sIgE to aero allergens, 4yrs	4				
IL-8, 6mo	1				E
IL-6, 6mo	1				
Eosinophils, 18mo	2				
sIgE max aero allergens, 6yrs	6				E
sIgE max aero allergens, 4yrs	4				E
TNF-α 6mo	1				
Eosinophils, 4yrs	4				
Total IgE, 4yrs	4				

Sensitization to aeroallergens, 4yrs	4				
DGGE score[+], 1 mo	0				
DGGE score[+], 1 yr	1				
Respiratory factors					
Neonatal FEV 0.5	0				
Methacholine (PD15), 1mo	0				E
Neonatal FEF 0.5	0				
sRaw, 4yrs	4				
sRaw, 7yrs	7				
FEV1, 7yrs	7				
FVC, 7yrs	7				
FEV1/FVC-index, 7yrs	7				
MMEF, 7yrs	7				
Exercise test, 7yrs	7				
Methacholine challenge test, 6 yrs	6				E
Cold air provocation at 6½ years	7				
Reversibility, spirometry, 5 yrs	5				E
Reversibility, sRaw, 5 yrs	5				

P = Pregnancy, * = Time to event, E = Excluded, $^{+}$ = a measure of gut microbial diversity in stool samples collected at 1 month of age and 1 year, assessed using Denaturing Gradient Gel Electrophoresis (DGGE). Higher scores indicate greater microbial diversity.

Data processing details

The initial dataset included 411 children and 113 variables. articipants with more than 50% missing data were excluded, reducing the dataset to 390 participants. Next, variables with low variance (n=11) were identified and removed, reducing the number of variables from 113 to 102. The excluded variables were: Methacholine at 1 month, IL-8 at 6 months, maternal aeroallergen-specific IgE maximum, sum of food allergen-specific IgE at 4 years, paternal total IgE, nicotine in hair, EPX:creatinine ratio at 4 weeks, Fel d 1 in dust in child's bedroom at 1 yr, NO in child's bedroom at 1 yr, total IgE at 6 years, and formaldehyde in child's bedroom at 1 yr

Following this, variables with greater than 90% correlation were identified (n=15) and removed, further reducing the variable set from 102 to 87. The excluded variables in this step included: Fat in the android region, fat in the trunk region, total body lean mass, cesarean delivery (Y/N), dog exposure in the first year (Y/N), cat exposure in the first year (Y/N), food allergen-specific IgE maximum at 6 years, food allergen-specific IgE maximum at 4 years, aeroallergen-specific IgE maximum at 6 years, aeroallergen-specific IgE maximum at 4 years, sum of aeroallergen-specific IgE for the mother, aeroallergen-specific IgE maximum for the father, alcohol intake (Y/N), smoking during pregnancy (Y/N), paternal education at 6 years.

Finally, variables with more than 33% total missingness were excluded (n=4), resulting in the removal of four additional variables and reducing the dataset to 83 variables. These removed variables were: Fat in the gynoid region, waist circumference at 6 years, methacholine at 6 years, and spirometry reversibility at 5 years.

The missing data at low missingness was imputed using iterative imputation.

Component-Resolved and Whole-Extract Pet Allergen Sensitization Show Similar Associations With Airway Inflammation and Bronchial Hyperresponsiveness at 18 Years

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Conflict of interest: AMS: Advisory board for ALK and paid speaker for ALK, Stallergenes Greer, and ThermoFisher Scientific. BC: Advisory board for ALK and paid speaker for ALK. KB: Advisory board for ALK. All other authors declare no potential, perceived, or real conflict of interest regarding the content of this manuscript. The funding agencies did not have any role in design and conduct of the study; collection, management, and interpretation of the data; or preparation, review, or approval of the manuscript. No pharmaceutical company was involved in the study.

Governance: We are aware of and comply with recognized codes of good research practice, including the Danish Code of Conduct for Research Integrity. We comply with national and international rules on the safety and rights of patients and healthy subjects, including Good Clinical Practice (GCP) as defined in the EU's Directive on Good Clinical Practice, the International Conference on Harmonisation's (ICH) good clinical practice guidelines and the Helsinki Declaration. Privacy is important to us which is why we follow national and international legislation on General Data Protection Regulation (GDPR), the Danish Act on Processing of Personal Data and the practice of the Danish Data Inspectorate.

Abbreviations:

AR allergic rhinitis

BHR bronchial hyperresponsiveness

COPSAC2000 Copenhagen Prospective Studies on Asthma in Childhood 2000

CRD component-resolved diagnostics

FeNO fractional exhaled nitric oxide

FEV_1 forced expiratory volume in 1 second

FVC forced vital capacity

HDM house dust mite

ICS inhaled corticosteroids

ISAC ImmunoCAP ISAC microarray

ISU ISAC Standardized Units

MMEF maximal mid-expiratory flow

PD_{20} provocative dose causing a 20% fall in FEV_1

sIgE specific immunoglobulin E

sRaw specific airway resistance

SPT skin prick test

T2 type 2

Abstract

Background: Component-resolved diagnostics allow measurement of IgE to individual pet allergen molecules, but it is unclear whether molecular sensitization patterns are more closely related to airway inflammation, bronchial hyperresponsiveness, and lung function than conventional whole-extract IgE.

Objective: To characterize molecular pet allergen sensitization at 18 years and examine its associations with airway inflammation, bronchial hyperresponsiveness, lung function, and current asthma.

Methods: In 347 adolescents from the population-based $COPSAC_{2000}$ cohort, whole-extract IgE to cat and dog, and molecular sensitization measured by ImmunoCAP ISAC were assessed at 18 years. Associations with spirometry, specific airway resistance (sRaw), fractional exhaled nitric oxide (FeNO), methacholine responsiveness (PD_{20}), and current asthma were analyzed using regression models adjusted for smoking, pet exposure, and house dust mite sensitization.

Results: Twenty-one percent were sensitized to at least one pet allergen component, most commonly Fel d 1. Sensitization - defined by either whole-extract IgE or molecular components - was not associated with spirometric indices or sRaw. In contrast, several components (Fel d 1, Fel d 4, Can f 5, Can f 6, and Equ c 1) and whole-extract IgE were associated with higher FeNO and lower PD_{20}, indicating increased airway inflammation and bronchial responsiveness. Molecular sensitization showed similar effect patterns to whole-extract IgE and did not provide consistent additional explanatory value. Polysensitization was associated with current asthma but not with greater physiological impairment.

Conclusion: In late adolescence, molecular and whole-extract pet sensitization show comparable associations with airway inflammation and bronchial responsiveness, while neither is linked to reduced baseline lung function. Component testing did not add substantial information beyond whole-extract IgE for airway outcomes at 18 years.

Background:

Sensitization to furry animals is common in childhood and adolescence, with prevalence increasing from early childhood into adolescence (1). Studies using specific IgE (sIgE) to whole cat and dog allergens have linked sensitization with asthma development, persistent wheeze, and bronchial hyperresponsiveness (BHR), whereas associations with impaired lung function, have been less consistent (2–6)**.** This variability suggests heterogeneity within pet allergen sensitization that may not be captured by whole-allergen IgE measurements alone.

In clinical practice, sensitization to pets is primarily assessed using skin prick testing (SPT) and specific IgE measurements to whole allergen extracts (7). As these extracts contain multiple allergenic proteins, testing reflects the aggregate IgE response and does not distinguish between sensitization to individual molecular components or cross-reactive molecules (8). Thus, whole-extract IgE may represent primary sensitization to a dominant species-specific allergen or reflect cross-reactive IgE responses to homologous proteins shared across mammalian species, such as lipocalins or serum albumins, a distinction that may be relevant when interpreting sensitization patterns, particularly in polysensitized adolescents (2,8,9). As a result, individuals with similar whole-extract sensitization profiles may differ substantially in their underlying molecular sensitization patterns.

Component-resolved diagnostics (CRD), including the ImmunoCAP ISAC 112 (8,10), enable measurement of IgE to individual pet allergen components and have revealed substantial heterogeneity within pet allergy. Cat sensitization is often dominated by Fel d 1, whereas dog sensitization involves more diverse molecular patterns (8,11–13). These approaches allow detailed characterization of mono- and poly-sensitization patterns that are not discernible from whole-extract testing alone (8,12).

Although previous studies have examined whole-extract and component-based sensitization in relation to airway inflammation and bronchial hyperresponsiveness (3,4), comprehensive comparisons across

individual components, broader sensitization patterns, and detailed objective airway outcomes in population-based adolescent cohorts remain limited.

Using data from the Copenhagen Prospective Studies on Asthma in Childhood 2000 ($COPSAC_{2000}$) cohort, we therefore examined how molecular component sensitization in late adolescence relates to whole-extract IgE, SPT reactivity, clinical symptoms, and objective measures of lung function, airway inflammation, BHR, and asthma at 18 years of age. We hypothesized that pet allergen sensitization is molecularly heterogeneous and that CRD provides complementary information beyond whole-extract IgE in relation to objective airway outcomes.

Methods:

The $COPSAC_{2000}$ cohort is a single-center prospective mother-child cohort study encompassing 411 children born to mothers with asthma. Infants were enrolled during their first month of life and followed with scheduled and acute clinical visits throughout childhood. The cohort, including the 18-year follow-up examination, has previously been described in detail (14–17). The present study is based on data obtained at the 18-year follow-up visit.

Ethics

The Copenhagen Ethics Committee (KF 01–289/96) and The Danish Data Protection Agency (2015-41-3696) approved the study. Oral and written informed consent were obtained from both parents at the time of enrollment, with the study participants themselves providing consent at the 18-year follow-up.

Objective assessments

From the 18-year visit, we included the following measurements:

Sensitization

Aeroallergen sensitization defined as sIgE ≥ 0.35 kUA/L (ImmunoCAP, Phadiatop InfantTM; Pharmacia Diagnostics AB, Uppsala, Sweden) and/or a positive skin prick test (wheal diameter ≥3 mm above negative control) to the allergen extract (Soluprick® SQ; ALK Abello) (15). We measured whole-extract sIgE to dog (e5) and cat (e1), and performed SPTs for horse, dog, and cat.

ISAC molecular component measurements were performed in participants with a positive Phadiatop screening result (sIgE ≥0.35 kUA/L). Results were reported in ISAC Standardized Units (ISU), the semi-quantitative scale used in the ImmunoCAP ISAC 112 microarray (Thermo Fisher Scientific, Uppsala, Sweden) (10). Component sensitization was defined as ISU ≥0.30 (10). The following pet allergen components were included in the analysis:

- Dog components: Can f1, Can f2, Can f3, Can f 4, Can f5, and Can f 6
- Cat components: Fel d1, Fel d 2, and Fel d4
- Horse components: Equ c1 and Equ c3

Allergen components with very low prevalence, defined as fewer than five observations (e.g. Fel d 2, Can f 2–4, Equ c 3), were excluded from correlation and regression analyses to ensure adequate analytical power. We did not have corresponding sIgE or skin prick test measurements for horse allergen extracts.

Binary sensitization variables (≥0.30 ISU) were used for all primary analyses. Continuous ISAC values were evaluated in supplementary analyses.

Lung function, airway inflammation and bronchial hyperresponsiveness

Specific airway resistance (sRaw) was assessed using whole-body plethysmography (MasterScreen Body; Jaeger, Würzburg, Germany), and spirometry (MasterScope system spirometer (Erich Jäeger, Würtzburg, Germany)) was performed according to guidelines using standardized calibration and reproducibility criteria (18,19). Forced expiratory volume in 1 second (FEV_1), maximal mid-expiratory flow (MMEF) and forced vital capacity (FVC) were derived from spirometry. BHR was assessed using methacholine challenge, with PD_{20} defined as the cumulative dose causing a 20 percent fall in FEV_1 (20). Fraction of exhaled nitric oxide (FeNO) was measured using the NIOX Flex device (Aerocrine, Solna, Sweden) before spirometry (21). Lung function measures were calibrated for age, sex, height, and respiratory infection status; FeNO was calibrated for recent infection.

Asthma and allergic rhinitis

Asthma was diagnosed based on recurrent troublesome lung symptoms, response to inhaled short-acting β_2-agonists and a course of inhaled corticosteroids (ICS) as described previously (14,22). Objective

confirmation of asthma required fulfilment of ≥2 of the following criteria at any clinical visit, in accordance with contemporary European Respiratory Society's recommendations for asthma diagnosis for children and adolescents (23):

(1) Obstructive lung function (FEV1 <80% or FEV1/FVC <80% measured by spirometry, and/or sRaw >1.6 kPa/s),

(2) A positive bronchodilator reversibility test (≥12% improvement in FEV1 or sRaw ≥30% after inhalation of a β2-agonist),

(3) Airway inflammation (FeNO ≥25 ppb), and/or

(4) Bronchial responsiveness to methacholine (drop in FEV1 ≥20% from baseline with a dose of methacholine ≤8 mg/mL) and/or exercise (≥10% decrease in FEV1).

Allergic rhinitis (AR) was defined based on self-reported symptoms of sneezing, nasal obstruction, rhinorrhea, or itchy/watery eyes upon exposure to cat, dog, or horse, combined with sensitization to the corresponding allergen (sIgE ≥0.35 kUA/L and/or SPT≥3 mm) (24).

Participants who had previously received an asthma diagnosis but did not fulfil the predefined objective confirmation criteria were excluded from asthma analyses.

Covariates

Demographic and exposure variables were obtained from the 18-year clinical assessment (16). Smoking status was self-reported and categorized descriptively; in regression analyses, smoking was included as a dichotomous variable (current smoker vs. non-current smoker). Current household exposure to cat and dog at 18 years was assessed and included as a species-specific covariate in models evaluating sensitization to the corresponding animal.

Sensitization to house dust mite (HDM; Dermatophagoides pteronyssinus, d1) was included in adjusted models to account for general atopic predisposition and potential co-sensitization.

All covariates were selected a priori based on clinical relevance and were included in multivariable models irrespective of statistical significance.

Statistical methods

The distribution and overlap of ISAC pet allergen component sensitization were visualized using UpSet plots. For these plots, ISAC results were dichotomized as sensitized or not sensitized to illustrate co-sensitization patterns across individuals. Differences in sensitization prevalence between adolescents with and without current asthma were assessed using Fisher's exact test due to low expected cell counts for several allergen components.

For correlation analyses and heatmap visualization, ISAC component ISU values, whole-extract sIgE levels, and SPT wheal sizes were analyzed as continuous measures, while AR variables were included as binary outcomes. Spearman correlations were computed to characterize the relationships between molecular sensitization, whole-extract IgE, skin prick test reactivity, and AR symptoms, as well as the degree of overlap with house dust mite (HDM) sensitization. As correlation magnitude is context dependent, coefficients were interpreted comparatively, with emphasis on relative differences in correlation patterns rather than absolute thresholds. Correlations were presented as a heatmap to illustrate co-sensitization patterns across allergen modalities.

Linear regression models were applied to examine associations between binary whole-extract sIgE sensitization (≥0.35 kU/L) and ISAC component sensitization (≥0.30 ISU) and calibrated lung function outcomes (FEV_1, MMEF, sRaw), airway inflammation (FeNO), and BHR (PD_{20}). Asthma at 18 years was analyzed as a binary outcome using logistic regression with the same exposure definitions and covariate adjustment strategy.

Due to skewed distributions, sRaw, FeNO, and PD_{20} were analyzed on the $\log_2$ scale, and back-transformed estimates are presented as fold-changes.

All regression models were adjusted for current smoking and current exposure to the corresponding animal. To evaluate the potential influence of co-sensitization between pet allergens and HDM, models were additionally fitted with and without adjustment for HDM sensitization. Monosensitization versus polysensitization was evaluated as a separate comparison using the same adjustment strategy.

To further assess robustness, additional sensitivity analyses were conducted using continuous ISAC component levels ($\log_2(\text{ISAC} + 0.1)$) instead of binary sensitization variables.

The regression models were also repeated separately among adolescents without current asthma and among those with current asthma at 18 years, using identical model specifications. These analyses were performed to explore whether associations between pet sensitization and respiratory outcomes differed according to asthma status and to assess potential subclinical effects in the non-asthma group.

Formal interaction between sensitization and asthma status was evaluated by including product terms in the regression models. Statistical significance of interaction effects was assessed using Wald tests of the interaction coefficients.

To evaluate whether molecular component sensitization provided additional information beyond whole-extract sensitization, nested regression models were constructed for each animal species. Base models included whole-extract sensitization, while extended models additionally included relevant molecular components. Model fit was compared using nested model comparisons. All models were adjusted for HDM sensitization, current smoking, and current exposure to the corresponding animal.

Given the hypothesis-driven design with pre-specified respiratory outcomes, no multiplicity correction was applied to the main regression analyses. False discovery rate (FDR) correction (Benjamini–Hochberg

procedure) was applied to nested model comparisons evaluating incremental value beyond whole-extract sensitization and to interaction analyses (25).

Statistical significance was set at $p < 0.05$ for all tests (two-sided). All analyses were performed using R (version 4.4.2).

Results:

A total of 347 (84%) of the 411 original cohort participants attended the 18-year follow-up visit and provided a blood sample, enabling assessment of whole-extract sIgE and ISAC component sensitization. The mean age at visit was 17.7 (SD 0.6) years, and 51% were female. Current asthma was present in 89 adolescents (30%). Overall, 73 individuals (21%) were sensitized to at least one selected pet allergen component. Baseline characteristics are presented in **Table 1**.

Sensitization prevalence by current asthma status is shown in **Table 2**. Whole-extract sensitization to cat (e1) and dog (e5) was more frequent among adolescents with current asthma (both $p < 0.001$). Similarly, sensitization to the major components Fel d 1, Can f 1, and Can f 5 was significantly more common in the asthma group, whereas less prevalent components showed no clear group differences.

Fel d 1 was the dominant component, observed in 63 individuals (86% of sensitized participants) (**Figure 1**). Monosensitization was present in 47 (64%), most commonly to Fel d 1 alone (n = 41), while 26 (36%) were polysensitized.

Correlations between pet and HDM sensitization

Spearman correlation are shown in **Figure 2**. Whole-extract sIgE to cat (e1) and dog (e5) were very strongly correlated (r = 0.91), and HDM sIgE (d1) correlated strongly with both pet extracts (e5, r = 0.83; e1, r = 0.77). In contrast, correlations between d1 and the molecular pet components were generally weaker.

ISAC Components and Rhinitis Symptoms

Correlations between ISAC components and species-specific rhinitis symptoms are shown in **Figure 2**. Fel d 1 showed the strongest correlation with cat-related rhinitis (r = 0.57), and among the dog

components, Can f 1 and Can f 6 showed the highest correlation with dog-related rhinitis (r = 0.36 and 0.38). For horse allergens, Equ c 1 correlated with horse-related rhinitis symptoms (r = 0.52).

Skin prick tests generally showed slightly higher or similar correlations with symptoms compared with ISAC components, whereas whole-extract sIgE demonstrated comparable or somewhat weaker correlations. Overall, the key ISAC components reflected rhinitis symptom patterns to a similar extent as whole-extract sIgE and SPT measures.

ISAC Components in Relation to sIgE and SPT

Across allergen groups, ISAC components correlated with their corresponding whole-extract sIgE levels and SPT, as shown in **Figure 2**. Fel d 1 and Can f 5 showed the highest correlations with both sIgE and SPT within the cat and dog groups, respectively, whereas components such as Fel d 4 and Can f 1 demonstrated lower correlation coefficients.

Lung function testing

In multivariable linear regression models adjusted for current smoking and animal exposure, pet sensitization - whether defined by whole-extract sIgE or molecular components - was not associated with baseline spirometric indices (FEV_1, FEV_1/FVC, or MMEF) or sRaw. Results were similar after additional adjustment for HDM sensitization (**Figure 3A+B**; **Supplementary Table S1**).

In contrast, sensitization was consistently associated with higher FeNO levels. After HDM adjustment, whole-extract sensitization to cat and dog remained significantly associated with elevated FeNO (cat: 1.39 [1.15; 1.69], $p < 0.001$; dog: 1.42 [1.17; 1.72], $p < 0.001$). At the molecular level, Fel d 1 and Fel d 4 were associated with 31% and 89% higher FeNO, respectively. Among dog components, Can f 5 and Can f 6 were associated with 53% and 89% higher FeNO, respectively. The strongest association among the

components was observed for Equ c 1, corresponding to a twofold higher FeNO (2.00 [1.42; 2.82], $p < 0.001$) (**Figure 3B**, **Supplementary Table S1**).

For BHR, several sensitizations were associated with significantly lower PD_{20} values, indicating increased airway responsiveness. After HDM adjustment, whole-extract cat sensitization (0.54 [0.32; 0.89], $p = 0.02$), Can f 1 (0.34 [0.11; 0.99], $p = 0.05$), Can f 6 (0.23 [0.07; 0.82], $p = 0.02$), and Equ c 1 (0.22 [0.08; 0.60], $p = 0.003$) remained significantly associated with reduced PD_{20}, whereas associations for other components were attenuated (**Figure 3B**, **Supplementary Table S1**).

In logistic regression analyses, whole-extract sensitization to cat and dog was associated with increased odds of current asthma after adjustment for HDM sensitization (cat: OR 2.44 [1.28; 4.67], $p = 0.01$; dog: OR 3.34 [1.76; 6.38], $p < 0.001$).

At the molecular level, sensitization to Can f 1 showed the strongest association with current asthma (OR 11.94 [2.95; 80.18], $p = 0.002$). Sensitization to Can f 5 was also associated with increased odds of asthma (OR 3.35 [1.34; 8.69], $p = 0.01$), whereas associations for cat components were attenuated after HDM adjustment (**Figure 3C**, **Supplementary Table S1**).

Polysensitized individuals had higher odds of current asthma compared with monosensitized participants (OR 2.90 [1.09; 8.10], $p = 0.04$). No corresponding differences were observed between mono- and polysensitized participants for lung function, FeNO, or PD_{20} (**Figure 3C**, **Supplementary Table S1**).

Continuous ISAC analyses

When modelling ISAC component levels as continuous predictors, effect estimates were generally attenuated compared with binary sensitization models but showed similar directions of association (**Supplementary Table S2**). Associations with spirometric outcomes and sRaw remained close to null. Higher FeNO levels were observed for several whole-extract and molecular predictors, including Fel d 1,

Fel d 4, Can f 5, Can f 6, and Equ c 1, even after HDM adjustment. Selected predictors were also associated with lower PD_{20} values. For current asthma, odds ratios per log_2-unit increase were elevated for whole-extract cat and dog sIgE and for Can f 1.

Analyses stratified by asthma status

In analyses restricted to adolescents with current asthma (**Supplementary Table S3**), effect directions were generally consistent with those observed in the full cohort. Most estimates were with wide CI and did not remain statistically significant after HDM adjustment. Sensitization to Fel d 4 remained significantly associated with lower MMEF (−0.95 [−1.74; −0.16], p = 0.02) after HDM adjustment and was also associated with lower FEV_1/FVC (−0.09 [−0.16; −0.01], p = 0.03). Whole-extract dog sensitization remained associated with higher FeNO after HDM adjustment (1.57 [1.12; 2.19], p = 0.01).

In participants without current asthma (**Supplementary Table S4**), pet sensitization remained associated with higher FeNO levels across several whole-extract and molecular predictors even after HDM adjustment. Associations with spirometric indices and sRaw were not consistent across predictors, although Fel d 4 was associated with higher FEV_1/FVC and MMEF and lower sRaw after HDM adjustment, and dog sIgE and Can f 6 were associated with higher MMEF. Associations with PD_{20} were attenuated and generally not statistically significant after HDM adjustment.

Interaction analyses

Interaction analyses between pet sensitization and asthma status did not demonstrate effect modification for FeNO, PD_{20}, or FEV_1 (**Supplementary Table S5**).

Significant interactions were observed for selected molecular components in relation to spirometric outcomes. Sensitization to Fel d 4 was associated with significant interaction terms for FEV_1/FVC ($p < 0.001$), MMEF (p = 0.003), and sRaw (p = 0.02). Likewise, Can f 6 and Equ c 1 showed significant

interactions for FEV_1/FVC ($p = 0.03$ and $p = 0.02$, respectively) and MMEF ($p = 0.02$ and $p = 0.03$, respectively).

Incremental analyses

Incremental analyses are presented in **Supplementary Table S6**. Across spirometric indices (FEV_1, FEV_1/FVC, MMEF), sRaw, and PD_{20}, inclusion of individual allergen components did not improve model fit beyond whole-extract sensitization (all $q \geq 0.32$).

For FeNO, addition of Fel d 4 to cat sIgE ($p=0.05$) and Can f 6 to dog sIgE ($p=0.04$) was associated with improved model fit; however, neither association remained significant after FDR correction (both $q=0.12$).

For current asthma, inclusion of Can f 1 in addition to dog sIgE improved model fit ($p=0.02$), but this did not remain statistically significant after FDR correction ($q=0.09$).

Overall, molecular component sensitization did not provide consistent incremental value beyond whole-extract sIgE in relation to lung function, airway inflammation, bronchial hyperresponsiveness, or current asthma at 18 years.

Discussion

Primary findings

ISAC-based sensitization to pet allergen components was dominated by cat allergens, with Fel d 1 accounting for the majority of cases. Most sensitized participants were monosensitized, whereas polysensitization was less common. Sensitization to individual pet components was not associated with worse lung function (FEV_1, MMEF, sRaw), while sensitization to several components showed clear associations with higher airway inflammation (FeNO) and increased BHR (lower PD_{20}). These patterns were directionally consistent in continuous ISAC models and in asthma-restricted analyses.

Importantly, these associations largely paralleled those observed for whole-extract sensitization, and molecular component sensitization did not provide consistent incremental explanatory value beyond whole-extract sIgE for lung function, airway inflammation, BHR, or current asthma.

Strength and limitations

This study benefits from data from the extensively characterized $COPSAC_{2000}$ mother-child cohort, where children were followed prospectively from birth with standardized diagnostic algorithms, high follow-up rates, and objective assessments of lung function, airway inflammation, and sensitization. The availability of ImmunoCAP ISAC measurements adds detailed information on molecular sensitization patterns, allowing examination of component-level associations not captured by whole-extract sIgE or skin prick testing alone. Adjustment for HDM sensitization further strengthened the specificity of pet-related associations.

A limitation of this study is the low sensitization frequency of some ISAC components, which reduced statistical power and necessitated their exclusion. In addition, the lack of corresponding whole-extract sIgE or skin prick test measurements for horse allergens limited direct comparison between molecular and

whole-extract sensitization. Moreover, the ISAC panel does not include certain newer pet allergen components (e.g., Can f 7, Fel d 7, and Fel d 8). Furthermore, the cohort consists exclusively of children born to mothers with asthma, resulting in a relatively homogeneous high-risk population, which may constrain generalizability to broader populations. Finally, a substantial proportion of adolescents with asthma were treated with ICS at the 18-year visit, which may have reduced FeNO levels and bronchial responsiveness. As a result, the observed associations between pet component sensitization, airway inflammation, and BHR are likely to represent conservative estimates, particularly within the asthma subgroup.

Interpretation

In this cohort, pet sensitization - whether defined by whole-extract IgE or molecular components - was consistently associated with airway inflammation and BHR, but not with measurable differences in baseline spirometry. This pattern aligns with findings from the European Community Respiratory Health Survey, where sensitization to cat was associated with asthma and bronchial hyperresponsiveness, while reductions in FEV_1 were modest and mainly observed among individuals with asthma (5,6). Thus, even in large adult populations, allergic sensitization appears more strongly linked to airway reactivity than to marked impairment in baseline lung function.

Studies using molecular diagnostics point in the same direction. In a population-based adult cohort, sensitization to specific pet components - particularly Fel d 1 and Fel d 4 - was associated with higher FeNO and increased BHR (4). Likewise, in children with severe asthma, sensitization to multiple animal-derived components was linked to elevated FeNO, higher eosinophil counts, and greater BHR (3). Together, these findings suggest that pet sensitization is primarily reflected in markers of T2 airway inflammation and reactivity.

Our results extend these observations to late adolescence in a longitudinal birth cohort. All examined components showed effect estimates that were directionally consistent with whole-based sensitization. However, the incremental analyses demonstrated that adding individual components to models including whole-extract IgE did not consistently improve model fit after correction for multiple testing. This indicates that, at age 18, the physiological signal captured by component testing largely overlaps with that identified by whole-extract IgE.

One possible explanation is that, in adolescence, the presence of pet sensitization per se - rather than the exact molecular profile - drives airway inflammation and bronchial responsiveness. Although molecular testing provides a more detailed description of sensitization patterns, these distinctions may not translate into measurable differences in airway physiology in predominantly mild-to-moderate asthma, which characterizes most adolescents in the COPSAC cohort.

In severe childhood asthma populations, broader multi-component sensitization has been associated with higher inflammatory markers and more pronounced clinical features, and has been interpreted as a marker of more advanced allergic disease (2,3). In contrast, polysensitized adolescents in our cohort were more likely to have current asthma than monosensitized individuals, but they did not differ consistently in lung function, FeNO, or BHR. This suggests that, at this age, a broader molecular sensitization pattern may reflect increased likelihood of asthma rather than greater physiological severity at a single time point. Moreover, the overall extent of polysensitization in our cohort was modest, which may further limit the ability to detect clinically meaningful differences in airway physiology.

Clinically, these findings indicate that while pet sensitization is clearly linked to airway inflammation and asthma risk, molecular profiling does not appear to provide substantial additional information beyond whole-extract IgE for assessing lung function or airway reactivity in late adolescence. For clinicians, this supports continued reliance on conventional sensitization testing in routine assessment, while recognizing

that airway inflammation and bronchial responsiveness - not spirometry alone - may better reflect the physiological consequences of allergic sensitization (23).

Conclusion:

In this high-risk mother–child cohort, pet allergen sensitization at 18 years was dominated by monosensitization, most commonly to Fel d 1. Both whole-extract and molecular pet sensitization were consistently associated with markers of T2 airway inflammation and, for selected predictors, increased bronchial responsiveness, whereas no consistent associations were observed with baseline lung function. Molecular component sensitization did not provide consistent incremental explanatory value beyond whole-extract sIgE for lung function, airway inflammation, BHR, or current asthma.

Accordingly, our hypothesis that molecular component patterns would provide complementary information beyond whole-extract IgE was not supported in this adolescent setting. Although statistically significant interactions were observed for selected components in relation to spirometric outcomes and sRaw, these findings should be interpreted cautiously considering multiple testing and small numbers within individual component-positive strata.

Tables

Table 1. Baseline characteristics of the population with ISAC pet component measurements

Characteristic	N = 347
Sex	
Female	177 / 347 (51%)
Age at visit, years	17.7 (0.6)
BMI (kg / m^2)	23.0 (4.1)
Smoking status	
Never smoker	171 / 290 (59%)
Occasional smoker (≤ monthly)	48 / 290 (17%)
Weekly smoker (<10 cig/week)	3 / 290 (0.3%)
Frequent smoker (≥10 cig/week)	68 / 290 (23%)
Cat exposure at home ≥ 180 days/year	64 / 347 (18%)
Dog exposure at home ≥ 180 days/year	112 / 347 (32%)
Fel d 1 (cat), sensitized	63 / 347 (18%)
Fel d 2 (cat), sensitized	0 / 347 (0.0%)
Fel d 4 (cat), sensitized	10 / 347 (2.9%)
Can f 1 (dog), sensitized	11 / 347 (3.2%)
Can f 2 (dog), sensitized	2 / 347 (0.6%)
Can f 3 (dog), sensitized	0 / 347 (0.0%)
Can f 4 (dog), sensitized	3 / 347 (0.9%)
Can f 5 (dog), sensitized	22 / 347 (6.3%)
Can f 6 (dog), sensitized	11 / 347 (3.2%)
Equ c 1 (horse), sensitized	14 / 347 (4.0%)
Equ c 3 (horse), sensitized	0 / 347 (0.0%)
FEV1 (L)	3.89 (0.44)

FVC (L)	4.35 (0.50)
FEV1/FVC ratio	0.90 (0.08)
MMEF (L/s)	4.25 (0.94)
sRaw (kPa·s/L)	0.15 (0.35)
FeNO (ppb)	23 (22)
PD_{20} methacholine	1.40 (3.62)
Atopy at 18 years	
Asthma	89 / 347 (30%)
Current inhaled corticosteroid use, n (%)	37 / 89 (41 %)
Allergic rhinitis	132 / 347 (38%)
Eczema	9 / 347 (2.6%)

Continuous variables are presented as mean (standard deviation); categorical variables as n/N (%). Sensitization was defined as ImmunoCAP ISAC ≥0.30 ISAC Standardized Units (ISU) according to the manufacturer's recommended cut-off (Thermo Fisher Scientific, Uppsala, Sweden). Forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), the FEV_1/FVC ratio, maximal mid-expiratory flow (MMEF), and specific airway resistance (sRaw) were calibrated for age, sex, height, and current respiratory infection. Fractional exhaled nitric oxide (FeNO) values were infection-calibrated and are presented in parts per billion (ppb). The provocative dose of methacholine causing a 20% fall in FEV_1 (PD_{20}) was calculated as the cumulative dose causing a 20% decline in FEV_1

Table 2. Prevalence of whole-extract and molecular pet allergen sensitization according to current asthma status at 18 years.

	Asthma (n=89)	No asthma (n=242)	p-value
sIgE cat (e1)	29 / 89 (32.6%)	32 / 242 (13.2%)	**<0.001**
sIgE dog (e5)	31 / 89 (34.8%)	29 / 242 (12%)	**<0.001**
Can f1 (dog)	9 / 89 (10.1%)	2 / 242 (0.8%)	**<0.001**
Can f2 (dog)	1 / 89 (1.1%)	1 / 242 (0.4%)	0.466
Can f3 (dog)	0 / 89 (0%)	0 / 242 (0%)	NA
Can f4 (dog)	1 / 89 (1.1%)	2 / 242 (0.8%)	1
Can f5 (dog)	13 / 89 (14.6%)	9 / 242 (3.7%)	**0.002**
Can f6 (dog)	6 / 89 (6.7%)	5 / 242 (2.1%)	0.08
Equ c1 (horse)	7 / 89 (7.9%)	7 / 242 (2.9%)	0.06
Equ c3 (horse)	0 / 89 (0%)	0 / 242 (0%)	NA
Fel d1 (cat)	27 / 89 (30.3%)	34 / 242 (14%)	**0.001**
Fel d2 (cat)	0 / 89 (0%)	0 / 242 (0%)	NA
Fel d4 (cat)	5 / 89 (5.6%)	5 / 242 (2.1%)	0.14

Data are presented as n/N (%). Differences between groups were evaluated using Fisher's exact test due to small expected cell counts for several allergen components.

Figures

Figure 1

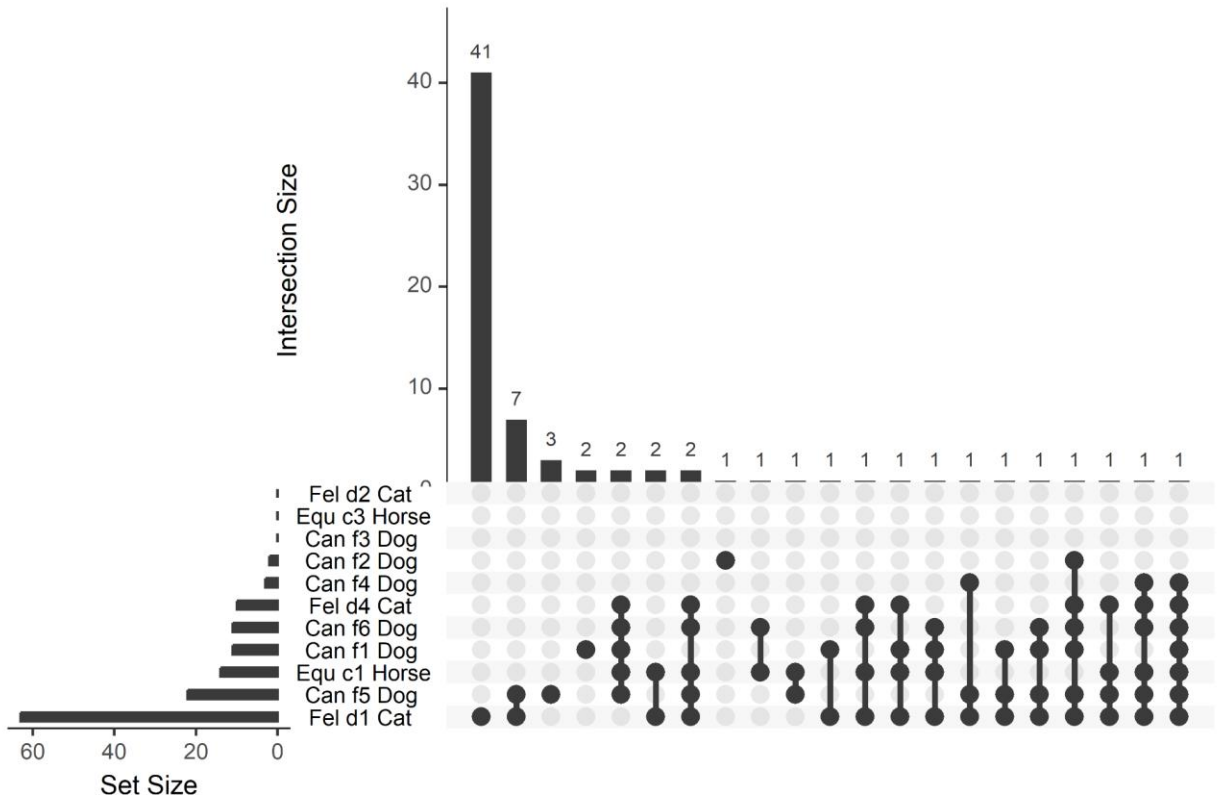


Figure 1: Upset plot illustrating the sensitization patterns to molecular allergen components measured using ISAC at 18 years. The vertical bars represent the intersection size, indicating the number of individuals sensitized to specific allergen combinations. The horizontal matrix shows individual allergen components, where filled black circles denote allergen co-sensitization patterns.

Figure 2

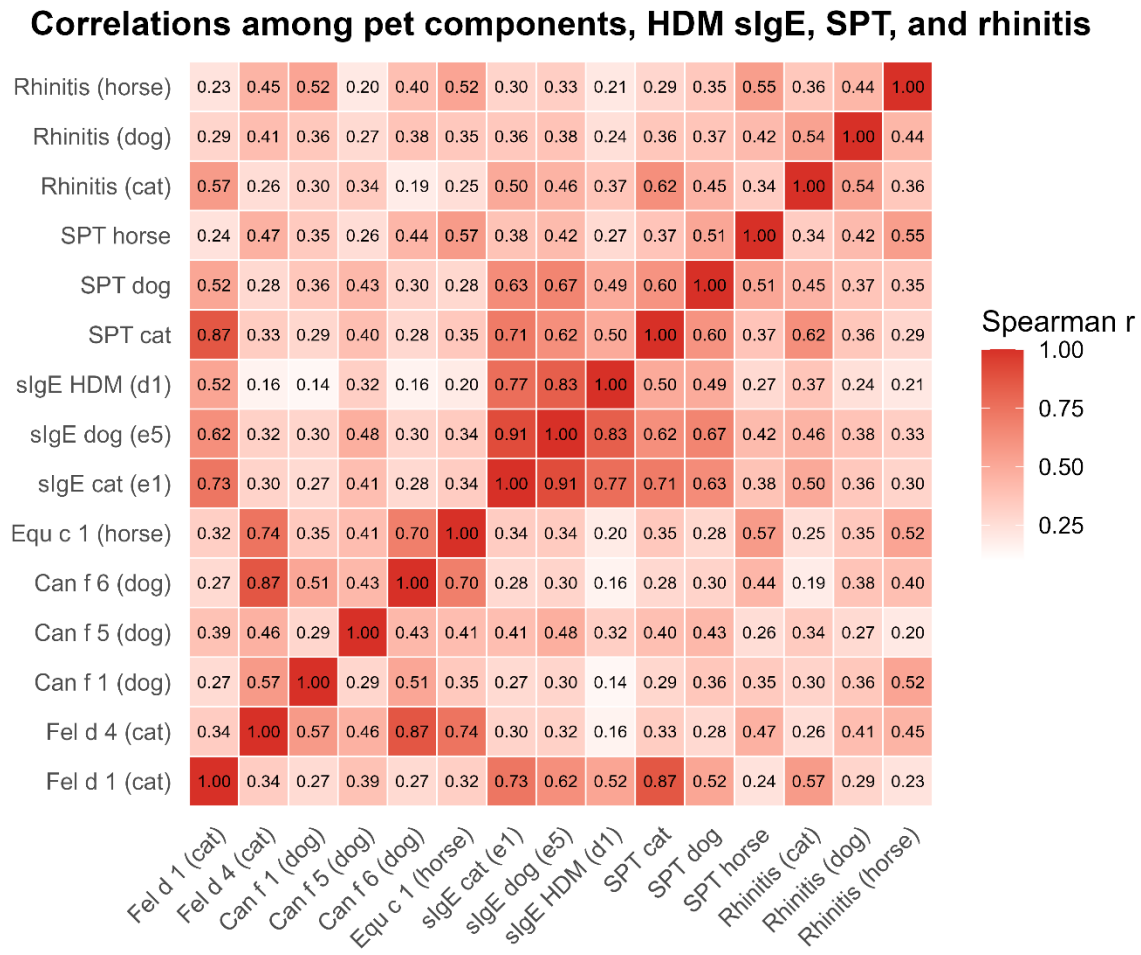
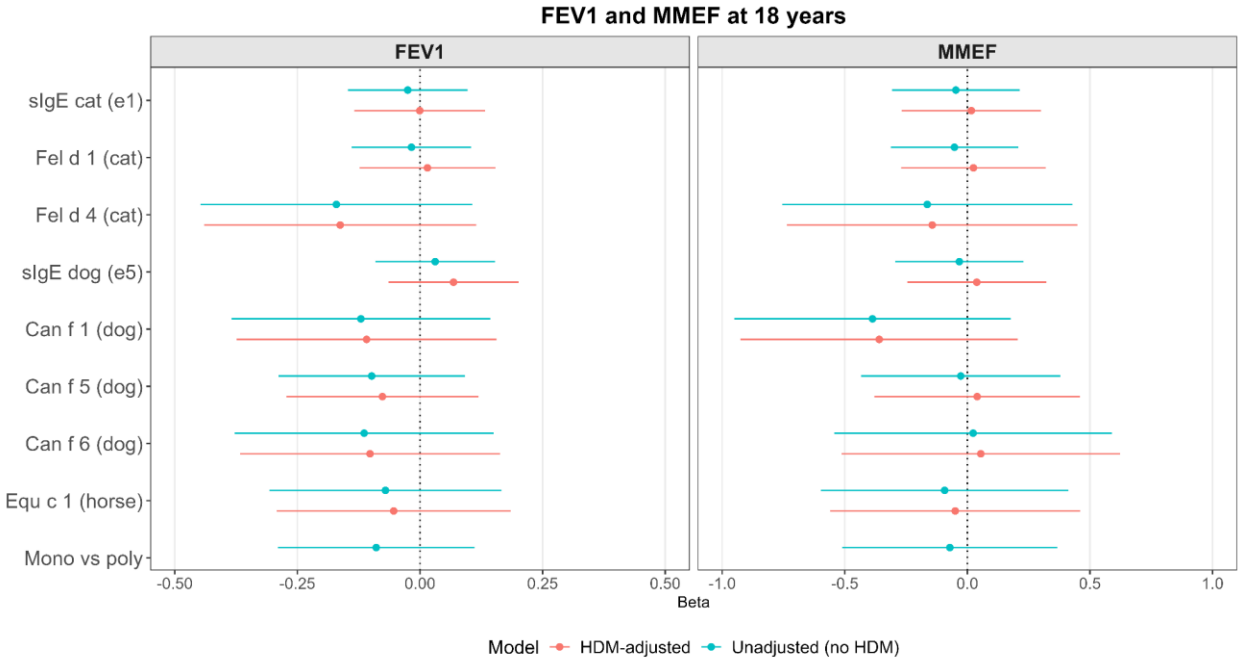


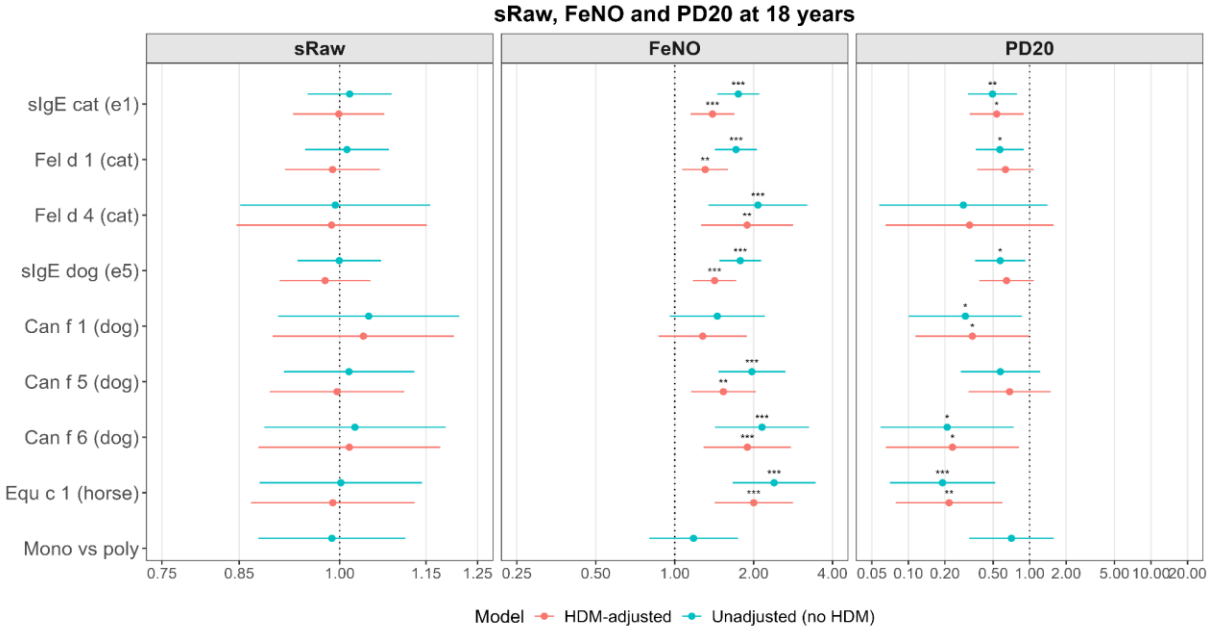
Figure 2: Heatmap showing Spearman correlation coefficients between molecular allergen sensitization (ISAC), specific IgE (sIgE), skin prick test (SPT), and allergic rhinitis (AR) symptoms at 18 years. Darker red indicates stronger positive correlations.

Figure 3

(A)


FEV1 and MMEF at 18 years
FEV1
MMEF
sIgE cat (e1)
Fel d 1 (cat)
Fel d 4 (cat)
sIgE dog (e5)
Can f 1 (dog)
Can f 5 (dog)
Can f 6 (dog)
Equ c 1 (horse)
Mono vs poly
-0.50
-0.25
0.00
0.25
0.50
-1.0
-0.5
0.0
0.5
1.0
Beta
Model
HDM-adjusted
Unadjusted (no HDM)

(B)


sRaw, FeNO and PD20 at 18 years
sRaw
FeNO
PD20
sIgE cat (e1)
Fel d 1 (cat)
Fel d 4 (cat)
sIgE dog (e5)
Can f 1 (dog)
Can f 5 (dog)
Can f 6 (dog)
Equ c 1 (horse)
Mono vs poly
0.75
0.85
1.00
1.15
1.25
0.25
0.50
1.00
2.00
4.00
0.05
0.10
0.20
0.50
1.00
2.00
5.00
10.00
20.00
Model
HDM-adjusted
Unadjusted (no HDM)

(C)

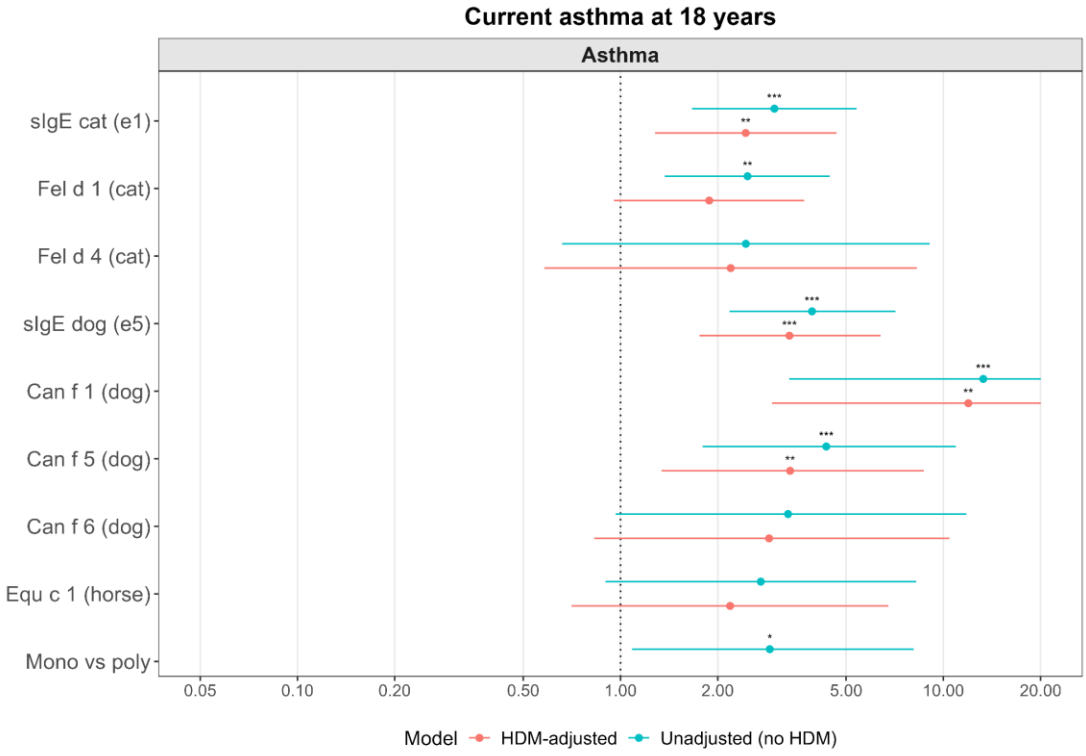


Figure 3. Forest plot of associations between pet allergen sensitization (ImmunoCAP ISAC standardized units (ISU) ≥0.30) and airway outcomes at 18 years.

(A) Forced expiratory volume in one second (FEV_1) and maximal mid-expiratory flow (MMEF), presented as β coefficients.
(B) Specific airway resistance (sRaw), fractional exhaled nitric oxide (FeNO), and provocative dose causing a 20% fall in FEV_1 (PD_{20}), presented as fold-changes from log_2-transformed models.
(C) Current asthma, presented as odds ratios.

Error bars represent 95% confidence intervals. The vertical line indicates no association (β = 0 or ratio/odds ratio = 1). Blue markers represent models adjusted for smoking and relevant pet exposure. Red markers represent models additionally adjusted for house dust mite sensitization. *p < 0.05; **p < 0.01; **p < 0.001.

Supplementary

Table S1. Associations between pet sensitization (binary) and lung function, airway inflammation, bronchial hyperresponsiveness, and current asthma at 18 years.

Outcome	Predictor	Smoking + animal exposure adjusted			Additionally adjusted for HDM		
		estimate	95% CI	p-value	estimate	95% CI	p-value
FEV1	sIgE cat (e1)	-0.03	[-0.15; 0.10]	0.69	0.00	[-0.13; 0.13]	0.99
	Fel d 1 (cat)	-0.02	[-0.14; 0.10]	0.78	0.02	[-0.12; 0.15]	0.83
	Fel d 4 (cat)	-0.17	[-0.45; 0.11]	0.23	-0.16	[-0.44; 0.11]	0.25
	sIgE dog (e5)	0.03	[-0.09; 0.15]	0.62	0.07	[-0.06; 0.20]	0.31
	Can f 1 (dog)	-0.12	[-0.38; 0.14]	0.37	-0.11	[-0.37; 0.16]	0.42
	Can f 5 (dog)	-0.10	[-0.29; 0.09]	0.31	-0.08	[-0.27; 0.12]	0.44
	Can f 6 (dog)	-0.11	[-0.38; 0.15]	0.40	-0.10	[-0.37; 0.16]	0.45
	Equ c 1 (horse)	-0.07	[-0.31; 0.17]	0.56	-0.05	[-0.29; 0.19]	0.66
	Mono vs poly	-0.09	[-0.29; 0.11]	0.38			
FEV1/FVC	sIgE cat (e1)	-0.01	[-0.03; 0.02]	0.59	0.00	[-0.02; 0.02]	0.88
	Fel d 1 (cat)	0.00	[-0.03; 0.02]	0.67	0.00	[-0.02; 0.02]	0.96
	Fel d 4 (cat)	-0.02	[-0.07; 0.02]	0.31	-0.02	[-0.07; 0.02]	0.34
	sIgE dog (e5)	-0.01	[-0.04; 0.01]	0.17	-0.01	[-0.04; 0.01]	0.30
	Can f 1 (dog)	-0.04	[-0.09; 0.00]	0.06	-0.04	[-0.09; 0.00]	0.07
	Can f 5 (dog)	0.00	[-0.03; 0.03]	1.00	0.00	[-0.03; 0.04]	0.77
	Can f 6 (dog)	-0.02	[-0.06; 0.03]	0.49	-0.01	[-0.06; 0.03]	0.54
	Equ c 1 (horse)	-0.03	[-0.07; 0.01]	0.17	-0.03	[-0.07; 0.02]	0.22
	Mono vs poly	-0.02	[-0.05; 0.02]	0.28			
MMEF	sIgE cat (e1)	-0.05	[-0.31; 0.21]	0.72	0.02	[-0.27; 0.30]	0.91
	Fel d 1 (cat)	-0.05	[-0.31; 0.21]	0.69	0.02	[-0.27; 0.32]	0.87
	Fel d 4 (cat)	-0.16	[-0.76; 0.43]	0.59	-0.14	[-0.74; 0.45]	0.64

	sIgE dog (e5)	-0.03	[-0.29; 0.23]	0.81	0.04	[-0.25; 0.32]	0.79
	Can f 1 (dog)	-0.39	[-0.95; 0.18]	0.18	-0.36	[-0.92; 0.21]	0.21
	Can f 5 (dog)	-0.03	[-0.43; 0.38]	0.90	0.04	[-0.38; 0.46]	0.85
	Can f 6 (dog)	0.02	[-0.54; 0.59]	0.94	0.05	[-0.51; 0.62]	0.85
	Equ c 1 (horse)	-0.09	[-0.60; 0.41]	0.72	-0.05	[-0.56; 0.46]	0.85
	Mono vs poly	-0.07	[-0.51; 0.37]	0.75			
sRaw	sIgE cat (e1)	1.02	[0.95; 1.09]	0.64	1.00	[0.93; 1.07]	0.97
	Fel d 1 (cat)	1.01	[0.95; 1.08]	0.73	0.99	[0.92; 1.07]	0.77
	Fel d 4 (cat)	0.99	[0.85; 1.16]	0.93	0.99	[0.85; 1.15]	0.87
	sIgE dog (e5)	1.00	[0.93; 1.07]	0.98	0.98	[0.91; 1.05]	0.53
	Can f 1 (dog)	1.05	[0.91; 1.21]	0.53	1.04	[0.90; 1.20]	0.61
	Can f 5 (dog)	1.02	[0.91; 1.13]	0.78	1.00	[0.89; 1.11]	0.94
	Can f 6 (dog)	1.02	[0.89; 1.19]	0.74	1.02	[0.88; 1.18]	0.83
	Equ c 1 (horse)	1.00	[0.88; 1.14]	0.98	0.99	[0.87; 1.13]	0.87
	Mono vs poly	0.99	[0.88; 1.11]	0.83			
FeNO	sIgE cat (e1)	1.75	[1.45; 2.10]	**<0.001**	1.39	[1.15; 1.69]	**<0.001**
	Fel d 1 (cat)	1.71	[1.42; 2.06]	**<0.001**	1.31	[1.07; 1.60]	**0.01**
	Fel d 4 (cat)	2.08	[1.35; 3.21]	**0.00**	1.89	[1.26; 2.83]	**0.00**
	sIgE dog (e5)	1.78	[1.48; 2.14]	**<0.001**	1.42	[1.17; 1.72]	**<0.001**
	Can f 1 (dog)	1.45	[0.96; 2.21]	0.08	1.28	[0.87; 1.88]	0.21
	Can f 5 (dog)	1.97	[1.47; 2.64]	**<0.001**	1.53	[1.15; 2.04]	**0.003**
	Can f 6 (dog)	2.15	[1.42; 3.25]	**<0.001**	1.89	[1.29; 2.78]	**0.001**
	Equ c 1 (horse)	2.39	[1.66; 3.45]	**<0.001**	2.00	[1.42; 2.82]	**<0.001**
	Mono vs poly	1.18	[0.80; 1.74]	0.40			
PD_{20}	sIgE cat (e1)	0.49	[0.31; 0.79]	**0.003**	0.54	[0.32; 0.89]	**0.02**
	Fel d 1 (cat)	0.57	[0.36; 0.90]	**0.02**	0.63	[0.37; 1.08]	0.09
	Fel d 4 (cat)	0.28	[0.06; 1.40]	0.12	0.32	[0.06; 1.57]	0.16
	sIgE dog (e5)	0.57	[0.36; 0.92]	**0.02**	0.65	[0.38; 1.08]	0.10

	Can f 1 (dog)	0.30	[0.10; 0.86]	**0.03**	0.34	[0.11; 0.99]	**0.05**
	Can f 5 (dog)	0.57	[0.27; 1.22]	0.15	0.68	[0.31; 1.49]	0.34
	Can f 6 (dog)	0.21	[0.06; 0.74]	**0.02**	0.23	[0.07; 0.82]	**0.02**
	Equ c 1 (horse)	0.19	[0.07; 0.52]	**0.00**	0.22	[0.08; 0.60]	**0.00**
	Mono vs poly	0.71	[0.32; 1.58]	0.39			
Current asthma	sIgE cat (e1)	3.00	[1.67; 5.39]	**<0.001**	2.44	[1.28; 4.67]	0.01
	Fel d 1 (cat)	2.47	[1.37; 4.44]	**0.00**	1.88	[0.95; 3.71]	0.07
	Fel d 4 (cat)	2.44	[0.66; 9.07]	0.17	2.19	[0.58; 8.28]	0.23
	sIgE dog (e5)	3.92	[2.18; 7.10]	**<0.001**	3.34	[1.76; 6.38]	**<0.001**
	Can f 1 (dog)	13.28	[3.33; 88.48]	**0.00**	11.94	[2.95; 80.18]	**0.00**
	Can f 5 (dog)	4.34	[1.80; 10.92]	**0.00**	3.35	[1.34; 8.69]	**0.01**
	Can f 6 (dog)	3.30	[0.97; 11.78]	0.05	2.89	[0.83; 10.44]	0.09
	Equ c 1 (horse)	2.72	[0.90; 8.23]	0.07	2.19	[0.71; 6.75]	0.17
	Mono vs poly	2.90	[1.09; 8.10]	**0.04**			

FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity; MMEF: maximal mid-expiratory flow; sRaw: specific airway resistance; FeNO: fractional exhaled nitric oxide; PD_{20}: provocative dose causing a 20% decline in FEV_1.

Linear regression models were applied for continuous outcomes and logistic regression for current asthma. sRaw, FeNO, and PD_{20} were analyzed on the $\log_2$ scale and are presented as fold-changes.

All models were adjusted for current smoking and current exposure to the corresponding animal. Models labelled “HDM-adjusted” were additionally adjusted for house dust mite (Dermatophagoides pteronyssinus, d1) sensitization.

Table S2. Associations between pet sensitization (continous) and lung function, airway inflammation, bronchial hyperresponsiveness, and current asthma at 18 years.

Outcome	Predictor	Smoking + animal exposure adjusted			Additionally adjusted for HDM		
		estimate	95% CI	p-value	estimate	95% CI	p-value
FEV1	sIgE cat (e1)	-0.02	[-0.06; 0.03]	0.48	-0.01	[-0.06; 0.04]	0.72
	Fel d 1 (cat)	-0.01	[-0.05; 0.03]	0.65	0.00	[-0.05; 0.05]	0.92
	Fel d 4 (cat)	-0.06	[-0.20; 0.08]	0.37	-0.06	[-0.20; 0.08]	0.43
	sIgE dog (e5)	-0.02	[-0.08; 0.05]	0.58	-0.01	[-0.08; 0.06]	0.85
	Can f 1 (dog)	-0.06	[-0.19; 0.06]	0.32	-0.06	[-0.19; 0.07]	0.35
	Can f 5 (dog)	-0.03	[-0.14; 0.08]	0.57	-0.02	[-0.13; 0.09]	0.72
	Can f 6 (dog)	-0.06	[-0.15; 0.04]	0.27	-0.05	[-0.15; 0.05]	0.31
	Equ c 1 (horse)	-0.06	[-0.16; 0.04]	0.24	-0.05	[-0.15; 0.04]	0.29
FEV1/FVC	sIgE cat (e1)	-0.01	[-0.01; 0.00]	0.17	0.00	[-0.01; 0.00]	0.29
	Fel d 1 (cat)	0.00	[-0.01; 0.00]	0.42	0.00	[-0.01; 0.01]	0.64
	Fel d 4 (cat)	0.00	[-0.03; 0.02]	0.90	0.00	[-0.02; 0.02]	0.98
	sIgE dog (e5)	-0.01	[-0.02; 0.00]	0.09	-0.01	[-0.02; 0.00]	0.15
	Can f 1 (dog)	-0.02	[-0.04; 0.00]	0.13	-0.02	[-0.04; 0.01]	0.15
	Can f 5 (dog)	0.00	[-0.02; 0.02]	0.84	0.00	[-0.02; 0.02]	0.98
	Can f 6 (dog)	-0.01	[-0.03; 0.01]	0.28	-0.01	[-0.03; 0.01]	0.31
	Equ c 1 (horse)	0.00	[-0.02; 0.01]	0.69	0.00	[-0.02; 0.01]	0.79
MMEF	sIgE cat (e1)	-0.05	[-0.15; 0.04]	0.28	-0.04	[-0.14; 0.07]	0.48
	Fel d 1 (cat)	-0.03	[-0.12; 0.06]	0.53	-0.01	[-0.11; 0.09]	0.82
	Fel d 4 (cat)	0.05	[-0.25; 0.35]	0.74	0.07	[-0.23; 0.37]	0.65
	sIgE dog (e5)	-0.07	[-0.20; 0.07]	0.36	-0.04	[-0.19; 0.11]	0.59
	Can f 1 (dog)	-0.16	[-0.43; 0.11]	0.23	-0.16	[-0.42; 0.11]	0.26
	Can f 5 (dog)	0.01	[-0.22; 0.25]	0.92	0.04	[-0.19; 0.28]	0.72
	Can f 6 (dog)	-0.05	[-0.26; 0.16]	0.66	-0.04	[-0.25; 0.17]	0.72

	Equ c 1 (horse)	0.00	[-0.21; 0.20]	0.96	0.01	[-0.20; 0.22]	0.92
sRaw	sIgE cat (e1)	1.02	[0.99; 1.04]	0.17	1.01	[0.99; 1.04]	0.32
	Fel d 1 (cat)	1.01	[0.99; 1.04]	0.37	1.01	[0.98; 1.03]	0.64
	Fel d 4 (cat)	0.98	[0.91; 1.06]	0.62	0.98	[0.90; 1.05]	0.52
	sIgE dog (e5)	1.02	[0.99; 1.06]	0.19	1.02	[0.98; 1.06]	0.37
	Can f 1 (dog)	1.04	[0.97; 1.11]	0.28	1.04	[0.97; 1.11]	0.31
	Can f 5 (dog)	1.02	[0.96; 1.08]	0.50	1.01	[0.95; 1.08]	0.69
	Can f 6 (dog)	1.02	[0.97; 1.08]	0.48	1.02	[0.96; 1.07]	0.54
	Equ c 1 (horse)	1.00	[0.94; 1.05]	0.87	0.99	[0.94; 1.05]	0.74
FeNO	sIgE cat (e1)	1.27	[1.19; 1.36]	**<0.001**	1.18	[1.11; 1.27]	**<0.001**
	Fel d 1 (cat)	1.26	[1.18; 1.34]	**<0.001**	1.17	[1.09; 1.25]	**<0.001**
	Fel d 4 (cat)	1.54	[1.24; 1.92]	**<0.001**	1.42	[1.16; 1.75]	**<0.001**
	sIgE dog (e5)	1.38	[1.25; 1.52]	**<0.001**	1.24	[1.13; 1.37]	**<0.001**
	Can f 1 (dog)	1.07	[0.88; 1.31]	0.51	1.03	[0.86; 1.24]	0.75
	Can f 5 (dog)	1.42	[1.20; 1.69]	**<0.001**	1.26	[1.07; 1.49]	**0.01**
	Can f 6 (dog)	1.30	[1.12; 1.52]	**<0.001**	1.25	[1.09; 1.45]	**0.002**
	Equ c 1 (horse)	1.41	[1.21; 1.64]	**<0.001**	1.32	[1.14; 1.52]	**<0.001**
PD_{20}	sIgE cat (e1)	0.79	[0.66; 0.94]	**0.01**	0.82	[0.68; 0.99]	**0.04**
	Fel d 1 (cat)	0.79	[0.67; 0.94]	**0.01**	0.82	[0.68; 0.99]	**0.04**
	Fel d 4 (cat)	0.59	[0.29; 1.24]	0.16	0.64	[0.31; 1.33]	0.23
	sIgE dog (e5)	0.67	[0.51; 0.88]	**0.003**	0.71	[0.53; 0.94]	**0.02**
	Can f 1 (dog)	0.46	[0.22; 0.97]	**0.04**	0.50	[0.23; 1.06]	0.07
	Can f 5 (dog)	0.80	[0.52; 1.24]	0.32	0.88	[0.56; 1.37]	0.56
	Can f 6 (dog)	0.60	[0.36; 1.02]	**0.06**	0.63	[0.38; 1.07]	**0.09**
	Equ c 1 (horse)	0.57	[0.36; 0.90]	**0.02**	0.61	[0.38; 0.96]	**0.03**
Current asthma	sIgE cat (e1)	1.51	[1.22; 1.92]	**<0.001**	1.41	[1.12; 1.81]	**0.01**
	Fel d 1 (cat)	1.43	[1.16; 1.78]	**0.001**	1.32	[1.05; 1.67]	**0.02**
	Fel d 4 (cat)	1.16	[0.54; 2.24]	0.67	1.04	[0.48; 2.03]	0.92

	sIgE dog (e5)	1.88	[1.35; 2.74]	**<0.001**	1.69	[1.20; 2.48]	**0.004**
	Can f 1 (dog)	4.87	[1.83; 26.72]	**0.02**	4.32	[1.71; 22.84]	**0.02**
	Can f 5 (dog)	1.76	[1.05; 3.10]	**0.04**	1.53	[0.90; 2.70]	0.12
	Can f 6 (dog)	1.36	[0.85; 2.25]	0.19	1.31	[0.81; 2.16]	0.26
	Equ c 1 (horse)	1.26	[0.78; 2.00]	0.32	1.16	[0.71; 1.85]	0.53

FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity; MMEF: maximal mid-expiratory flow; sRaw: specific airway resistance; FeNO: fractional exhaled nitric oxide; PD_{20}: provocative dose causing a 20% decline in FEV_1.

Linear regression models were applied for continuous outcomes and logistic regression for current asthma. sRaw, FeNO, and PD_{20} were analyzed on the $\log_2$ scale and are presented as fold-changes.

All models were adjusted for current smoking and current exposure to the corresponding animal. Models labelled "HDM-adjusted" were additionally adjusted for house dust mite (Dermatophagoides pteronyssinus, d1) sensitization.

Table S3 Associations between pet sensitization (binary) and lung function, airway inflammation, bronchial hyperresponsiveness, and current asthma at 18 years in asthma population (n = 89)

Outcome	Predictor	Unadjusted			HDM-adjusted		
		estimate	95% CI	p-value	estimate	95% CI	p-value
FEV1	sIgE cat (e1)	-0.01	[-0.20; 0.18]	0.91	-0.02	[-0.22; 0.19]	0.88
	Fel d 1 (cat)	0.02	[-0.18; 0.22]	0.84	0.02	[-0.21; 0.25]	0.86
	Fel d 4 (cat)	-0.24	[-0.62; 0.15]	0.22	-0.25	[-0.64; 0.15]	0.22
	sIgE dog (e5)	0.08	[-0.11; 0.27]	0.39	0.08	[-0.11; 0.28]	0.41
	Can f 1 (dog)	-0.04	[-0.33; 0.25]	0.79	-0.04	[-0.33; 0.25]	0.80
	Can f 5 (dog)	0.00	[-0.25; 0.25]	0.99	-0.01	[-0.26; 0.25]	0.95
	Can f 6 (dog)	-0.09	[-0.44; 0.26]	0.60	-0.09	[-0.44; 0.26]	0.61
	Equ c 1 (horse)	-0.05	[-0.38; 0.28]	0.77	-0.05	[-0.38; 0.28]	0.77
	Mono vs poly	0.03	[-0.25; 0.31]	0.82			
FEV_1/FVC	sIgE cat (e1)	0.01	[-0.03; 0.04]	0.78	0.00	[-0.04; 0.04]	0.91
	Fel d 1 (cat)	0.01	[-0.03; 0.04]	0.77	0.00	[-0.04; 0.05]	0.94
	Fel d 4 (cat)	-0.09	[-0.16; -0.01]	0.02	-0.09	[-0.16; -0.01]	0.03
	sIgE dog (e5)	0.00	[-0.04; 0.04]	0.91	0.00	[-0.04; 0.04]	0.96
	Can f 1 (dog)	-0.02	[-0.07; 0.04]	0.56	-0.02	[-0.08; 0.04]	0.56
	Can f 5 (dog)	0.02	[-0.03; 0.07]	0.37	0.02	[-0.03; 0.07]	0.42
	Can f 6 (dog)	-0.04	[-0.11; 0.03]	0.24	-0.04	[-0.11; 0.03]	0.25
	Equ c 1 (horse)	-0.06	[-0.12; 0.00]	0.07	-0.06	[-0.12; 0.00]	0.07
	Mono vs poly	-0.03	[-0.08; 0.03]	0.35			
MMEF	sIgE cat (e1)	-0.02	[-0.42; 0.37]	0.90	-0.07	[-0.49; 0.36]	0.76

	Fel d 1 (cat)	0.05	[-0.35; 0.45]	0.81	0.01	[-0.46; 0.47]	0.98
	Fel d 4 (cat)	-0.94	[-1.70; -0.18]	**0.02**	-0.95	[-1.74; -0.16]	**0.02**
	sIgE dog (e5)	0.04	[-0.35; 0.43]	0.82	0.01	[-0.40; 0.42]	0.96
	Can f 1 (dog)	-0.08	[-0.69; 0.52]	0.78	-0.08	[-0.69; 0.52]	0.78
	Can f 5 (dog)	0.09	[-0.42; 0.61]	0.72	0.07	[-0.46; 0.59]	0.81
	Can f 6 (dog)	-0.38	[-1.10; 0.34]	0.30	-0.37	[-1.09; 0.35]	0.31
	Equ c 1 (horse)	-0.48	[-1.15; 0.19]	0.16	-0.48	[-1.15; 0.20]	0.16
	Mono vs poly	-0.11	[-0.71; 0.48]	0.70			
	sIgE cat (e1)	0.99	[0.89; 1.11]	0.89	1.00	[0.88; 1.12]	0.94
	Fel d 1 (cat)	0.96	[0.85; 1.07]	0.46	0.95	[0.84; 1.09]	0.46
	Fel d 4 (cat)	1.17	[0.94; 1.46]	0.16	1.17	[0.93; 1.48]	0.17
	sIgE dog (e5)	0.89	[0.80; 1.00]	0.05	0.89	[0.79; 1.00]	0.05
sRaw	Can f 1 (dog)	0.99	[0.83; 1.18]	0.92	0.99	[0.83; 1.18]	0.92
	Can f 5 (dog)	1.03	[0.88; 1.19]	0.73	1.03	[0.89; 1.20]	0.66
	Can f 6 (dog)	1.10	[0.89; 1.35]	0.38	1.09	[0.89; 1.35]	0.40
	Equ c 1 (horse)	1.03	[0.84; 1.25]	0.80	1.03	[0.84; 1.25]	0.80
	Mono vs poly	1.05	[0.87; 1.28]	0.60			
	sIgE cat (e1)	1.48	[1.04; 2.11]	**0.03**	1.18	[0.82; 1.68]	0.37
	Fel d 1 (cat)	1.52	[1.06; 2.18]	**0.02**	1.11	[0.75; 1.64]	0.61
	Fel d 4 (cat)	1.23	[0.59; 2.57]	0.58	1.71	[0.86; 3.39]	0.12
	sIgE dog (e5)	1.85	[1.32; 2.59]	**<0.001**	1.57	[1.12; 2.19]	**0.01**
FeNO	Can f 1 (dog)	1.23	[0.71; 2.15]	0.46	1.22	[0.74; 2.03]	0.43
	Can f 5 (dog)	1.59	[1.00; 2.54]	**0.05**	1.34	[0.86; 2.09]	0.19
	Can f 6 (dog)	1.27	[0.65; 2.47]	0.49	1.36	[0.74; 2.50]	0.32
	Equ c 1 (horse)	1.48	[0.79; 2.76]	0.22	1.48	[0.83; 2.62]	0.18
	Mono vs poly	0.93	[0.55; 1.59]	0.80			
PD_{20}	sIgE cat (e1)	0.55	[0.28; 1.10]	0.09	0.64	[0.29; 1.41]	0.27

	Fel d 1 (cat)	0.49	[0.25; 1.00]	**0.05**	0.56	[0.23; 1.36]	0.19
	Fel d 4 (cat)	0.24	[0.02; 2.87]	0.26	0.19	[0.02; 2.19]	0.18
	sIgE dog (e5)	0.53	[0.26; 1.08]	0.08	0.61	[0.29; 1.30]	0.20
	Can f 1 (dog)	0.42	[0.13; 1.36]	0.14	0.47	[0.15; 1.53]	0.21
	Can f 5 (dog)	0.91	[0.35; 2.32]	0.84	1.14	[0.43; 3.03]	0.79
	Can f 6 (dog)	0.20	[0.03; 1.21]	0.08	0.21	[0.04; 1.26]	0.09
	Equ c 1 (horse)	0.23	[0.06; 0.80]	**0.02**	0.26	[0.07; 0.94]	**0.04**
	Mono vs poly	1.04	[0.35; 3.14]	0.94			

Table S4 Children without asthma

Outcome	Predictor	Unadjusted			HDM-adjusted		
		estimate	95% CI	p-value	estimate	95% CI	p-value
FEV1	sIgE cat (e1)	0.04	[-0.12; 0.21]	0.62	0.08	[-0.10; 0.26]	0.38
	Fel d 1 (cat)	0.02	[-0.14; 0.18]	0.80	0.06	[-0.12; 0.24]	0.50
	Fel d 4 (cat)	-0.05	[-0.43; 0.34]	0.81	-0.01	[-0.41; 0.39]	0.95
	sIgE dog (e5)	0.09	[-0.08; 0.26]	0.31	0.14	[-0.05; 0.33]	0.14
	Can f 1 (dog)	0.13	[-0.48; 0.74]	0.68	0.17	[-0.45; 0.79]	0.59
	Can f 5 (dog)	-0.06	[-0.35; 0.23]	0.70	-0.03	[-0.33; 0.28]	0.87
	Can f 6 (dog)	-0.02	[-0.41; 0.37]	0.91	0.01	[-0.39; 0.40]	0.97
	Equ c 1 (horse)	-0.01	[-0.34; 0.32]	0.95	0.02	[-0.32; 0.36]	0.90
	Mono vs poly	-0.09	[-0.41; 0.22]	0.56			
FEV_1/FVC	sIgE cat (e1)	0.01	[-0.01; 0.04]	0.34	0.02	[-0.01; 0.05]	0.21
	Fel d 1 (cat)	0.01	[-0.01; 0.04]	0.36	0.02	[-0.01; 0.05]	0.20
	Fel d 4 (cat)	0.06	[-0.00; 0.12]	0.06	0.07	[0.00; 0.13]	**0.04**
	sIgE dog (e5)	0.01	[-0.02; 0.03]	0.65	0.01	[-0.02; 0.04]	0.46
	Can f 1 (dog)	0.03	[-0.07; 0.12]	0.56	0.03	[-0.06; 0.13]	0.51
	Can f 5 (dog)	0.02	[-0.02; 0.07]	0.29	0.03	[-0.02; 0.08]	0.21
	Can f 6 (dog)	0.05	[-0.01; 0.11]	0.09	0.06	[-0.00; 0.12]	0.07
	Equ c 1 (horse)	0.03	[-0.02; 0.08]	0.23	0.04	[-0.02; 0.09]	0.18
	Mono vs poly	0.02	[-0.02; 0.06]	0.32			
MMEF	sIgE cat (e1)	0.23	[-0.11; 0.57]	0.19	0.34	[-0.03; 0.71]	0.07

	Fel d 1 (cat)	0.11	[-0.22; 0.45]	0.50	0.22	[-0.15; 0.60]	0.24
	Fel d 4 (cat)	0.83	[0.02; 1.63]	**0.04**	0.95	[0.13; 1.78]	**0.02**
	sIgE dog (e5)	0.27	[-0.08; 0.63]	0.13	0.40	[0.01; 0.79]	**0.04**
	Can f 1 (dog)	0.43	[-0.85; 1.71]	0.51	0.52	[-0.77; 1.82]	0.43
	Can f 5 (dog)	0.41	[-0.20; 1.03]	0.18	0.53	[-0.11; 1.16]	0.10
	Can f 6 (dog)	0.92	[0.11; 1.73]	**0.03**	1.02	[0.20; 1.84]	**0.02**
	Equ c 1 (horse)	0.59	[-0.10; 1.28]	0.09	0.69	[-0.01; 1.39]	**0.05**
	Mono vs poly	0.44	[-0.18; 1.06]	0.16			
	sIgE cat (e1)	0.98	[0.90; 1.07]	0.67	0.96	[0.87; 1.05]	0.36
	Fel d 1 (cat)	1.00	[0.92; 1.09]	0.96	0.97	[0.88; 1.07]	0.55
	Fel d 4 (cat)	0.82	[0.67; 1.00]	**0.05**	0.79	[0.64; 0.97]	**0.03**
	sIgE dog (e5)	0.99	[0.90; 1.08]	0.78	0.96	[0.87; 1.06]	0.41
sRaw	Can f 1 (dog)	0.85	[0.62; 1.18]	0.34	0.83	[0.60; 1.15]	0.25
	Can f 5 (dog)	0.89	[0.76; 1.04]	0.13	0.86	[0.73; 1.00]	0.06
	Can f 6 (dog)	0.87	[0.71; 1.07]	0.19	0.85	[0.69; 1.05]	0.12
	Equ c 1 (horse)	0.92	[0.78; 1.10]	0.37	0.90	[0.75; 1.08]	0.24
	Mono vs poly	0.87	[0.74; 1.02]	0.08			
	sIgE cat (e1)	1.91	[1.51; 2.42]	**<0.001**	1.53	[1.19; 1.96]	**<0.001**
	Fel d 1 (cat)	1.81	[1.44; 2.28]	**<0.001**	1.40	[1.09; 1.80]	**0.01**
	Fel d 4 (cat)	3.15	[1.78; 5.59]	**<0.001**	2.21	[1.27; 3.85]	**0.01**
	sIgE dog (e5)	1.75	[1.36; 2.24]	**<0.001**	1.34	[1.03; 1.74]	**0.03**
FeNO	Can f 1 (dog)	1.62	[0.64; 4.08]	0.30	1.08	[0.45; 2.57]	0.86
	Can f 5 (dog)	2.31	[1.50; 3.54]	**<0.001**	1.67	[1.10; 2.55]	**0.02**
	Can f 6 (dog)	3.48	[1.97; 6.14]	**<0.001**	2.63	[1.53; 4.51]	**<0.001**
	Equ c 1 (horse)	3.43	[2.13; 5.52]	**<0.001**	2.59	[1.63; 4.11]	**<0.001**
	Mono vs poly	1.47	[0.77; 2.79]	0.24			
PD_{20}	sIgE cat (e1)	0.58	[0.31; 1.08]	0.09	0.62	[0.31; 1.23]	0.17

	Fel d 1 (cat)	0.73	[0.40; 1.34]	0.31	0.83	[0.42; 1.66]	0.60
	Fel d 4 (cat)	0.31	[0.04; 2.27]	0.25	0.37	[0.05; 2.78]	0.33
	sIgE dog (e5)	0.78	[0.41; 1.48]	0.45	0.90	[0.45; 1.81]	0.76
	Can f 1 (dog)	0.49	[0.06; 3.71]	0.48	0.59	[0.07; 4.62]	0.61
	Can f 5 (dog)	0.63	[0.19; 2.05]	0.44	0.73	[0.21; 2.47]	0.61
	Can f 6 (dog)	0.24	[0.05; 1.25]	0.09	0.26	[0.05; 1.40]	0.12
	Equ c 1 (horse)	0.22	[0.05; 0.92]	**0.04**	0.24	[0.06; 1.03]	0.06
	Mono vs poly	0.75	[0.21; 2.65]	0.64			

Table S5 Interactions

Outcome	Predictor	Smoking + animal exposure + HDM adjusted		
		estimate	95% CI	p-value
FEV1	sIgE cat (e1)	-0.04	[-0.29; 0.21]	0.75
	Fel d 1 (cat)	0.02	[-0.23; 0.27]	0.88
	Fel d 4 (cat)	-0.18	[-0.74; 0.38]	0.53
	sIgE dog (e5)	-0.03	[-0.28; 0.23]	0.84
	Can f 1 (dog)	-0.17	[-0.85; 0.5]	0.61
	Can f 5 (dog)	0.05	[-0.34; 0.44]	0.80
	Can f 6 (dog)	-0.08	[-0.61; 0.45]	0.77
	Equ c 1 (horse)	-0.03	[-0.5; 0.44]	0.91
FEV1/FVC	sIgE cat (e1)	0.00	[-0.05; 0.04]	0.86
	Fel d 1 (cat)	0.00	[-0.05; 0.04]	0.87
	Fel d 4 (cat)	-0.15	[-0.24; -0.05]	0.00
	sIgE dog (e5)	0.00	[-0.05; 0.04]	0.84
	Can f 1 (dog)	-0.05	[-0.16; 0.07]	0.42
	Can f 5 (dog)	0.00	[-0.07; 0.06]	0.92
	Can f 6 (dog)	-0.10	[-0.18; -0.01]	0.03
	Equ c 1 (horse)	-0.09	[-0.17; -0.01]	0.02
MMEF	sIgE cat (e1)	-0.21	[-0.74; 0.32]	0.43
	Fel d 1 (cat)	-0.02	[-0.55; 0.51]	0.94
	Fel d 4 (cat)	-1.78	[-2.93; -0.63]	**0.003**
	sIgE dog (e5)	-0.27	[-0.8; 0.27]	0.33
	Can f 1 (dog)	-0.54	[-1.95; 0.87]	0.45
	Can f 5 (dog)	-0.35	[-1.16; 0.45]	0.39
	Can f 6 (dog)	-1.35	[-2.45; -0.26]	**0.02**
	Equ c 1 (horse)	-1.09	[-2.06; -0.12]	**0.03**
sRaw	sIgE cat (e1)	0.99	[0.86; 1.14]	0.90

	Fel d 1 (cat)	0.94	[0.82; 1.08]	0.39
	Fel d 4 (cat)	1.42	[1.05; 1.93]	0.02
	sIgE dog (e5)	0.92	[0.8; 1.06]	0.23
	Can f 1 (dog)	1.18	[0.81; 1.7]	0.39
	Can f 5 (dog)	1.17	[0.95; 1.44]	0.15
	Can f 6 (dog)	1.28	[0.96; 1.7]	0.10
	Equ c 1 (horse)	1.12	[0.86; 1.44]	0.40
FeNO	sIgE cat (e1)	0.83	[0.58; 1.21]	0.34
	Fel d 1 (cat)	0.88	[0.6; 1.28]	0.50
	Fel d 4 (cat)	0.77	[0.34; 1.77]	0.54
	sIgE dog (e5)	1.14	[0.78; 1.66]	0.49
	Can f 1 (dog)	1.19	[0.43; 3.26]	0.73
	Can f 5 (dog)	0.83	[0.47; 1.46]	0.51
	Can f 6 (dog)	0.52	[0.24; 1.14]	0.10
	Equ c 1 (horse)	0.60	[0.3; 1.2]	0.15
PD_{20}	sIgE cat (e1)	0.97	[0.37; 2.58]	0.96
	Fel d 1 (cat)	0.75	[0.29; 1.96]	0.56
	Fel d 4 (cat)	0.60	[0.02; 17.01]	0.77
	sIgE dog (e5)	0.68	[0.25; 1.82]	0.44
	Can f 1 (dog)	0.78	[0.07; 8.08]	0.83
	Can f 5 (dog)	1.45	[0.32; 6.57]	0.63
	Can f 6 (dog)	0.87	[0.07; 10.96]	0.92
	Equ c 1 (horse)	1.13	[0.16; 8.02]	0.90

Table S6 Incremental analyses

Outcome	Predictor		Whole-extract effect			Component effect			Nested test (F/χ^2)	
			estimate	95% CI	p-value	estimate	95% CI	p-value	p	q
FEV1	sIgE cat	Fel d 1	-0.03	[-0.25; 0.19]	0.77	0.04	[-0.18; 0.27]	0.72	0.72	0.72
		Fel d 4	0.03	[-0.11; 0.18]	0.65	-0.19	[-0.49; 0.11]	0.22	0.22	0.27
	sIgE dog	Can f 1	0.11	[-0.04; 0.25]	0.15	-0.19	[-0.48; 0.09]	0.19	0.19	0.27
		Can f 5	0.11	[-0.03; 0.26]	0.13	-0.15	[-0.37; 0.07]	0.17	0.17	0.27
		Can f 6	0.10	[-0.04; 0.25]	0.16	-0.18	[-0.47; 0.10]	0.21	0.21	0.27
FEV1/ FVC	sIgE cat	Fel d 1	-0.01	[-0.04; 0.03]	0.75	0.01	[-0.03; 0.05]	0.77	0.77	0.82
		Fel d 4	0.00	[-0.02; 0.03]	0.83	-0.03	[-0.08; 0.03]	0.33	0.33	0.67
	sIgE dog	Can f 1	0.00	[-0.03; 0.02]	0.72	-0.04	[-0.09; 0.01]	0.13	0.13	0.66
		Can f 5	-0.02	[-0.04; 0.01]	0.19	0.02	[-0.02; 0.05]	0.40	0.40	0.67
		Can f 6	-0.01	[-0.04; 0.01]	0.39	-0.01	[-0.06; 0.04]	0.82	0.82	0.82
MMEF	sIgE cat	Fel d 1	-0.01	[-0.47; 0.46]	0.98	0.03	[-0.45; 0.52]	0.90	0.90	0.94
		Fel d 4	0.05	[-0.26; 0.35]	0.75	-0.18	[-0.82; 0.46]	0.58	0.58	0.94
	sIgE dog	Can f 1	0.13	[-0.18; 0.44]	0.41	-0.46	[-1.07; 0.15]	0.14	0.14	0.71
		Can f 5	0.03	[-0.28; 0.35]	0.84	0.02	[-0.45; 0.49]	0.94	0.94	0.94
		Can f 6	0.03	[-0.27; 0.34]	0.83	0.03	[-0.58; 0.64]	0.92	0.93	0.94
sRaw	sIgE cat	Fel d 1	0.03	[-0.15; 0.20]	0.75	-0.04	[-0.22; 0.14]	0.67	0.67	0.87
		Fel d 4	0.00	[-0.11; 0.12]	0.98	-0.02	[-0.26; 0.22]	0.87	0.87	0.87
	sIgE dog	Can f 1	-0.05	[-0.17; 0.06]	0.37	0.10	[-0.13; 0.33]	0.41	0.41	0.87
		Can f 5	-0.04	[-0.16; 0.08]	0.50	0.02	[-0.15; 0.20]	0.82	0.82	0.87
		Can f 6	-0.05	[-0.16; 0.07]	0.44	0.06	[-0.17; 0.29]	0.62	0.62	0.87
FeNO	sIgE cat	Fel d 1	0.50	[0.04; 0.95]	**0.03**	-0.02	[-0.50; 0.45]	0.93	0.93	0.93
		Fel d 4	0.37	[0.07; 0.66]	**0.02**	0.63	[0.01; 1.25]	**0.05**	**0.05**	0.12
	sIgE dog	Can f 1	0.52	[0.22; 0.81]	**<0.001**	-0.05	[-0.65; 0.55]	0.87	0.87	0.93
		Can f 5	0.40	[0.09; 0.71]	**0.01**	0.35	[-0.10; 0.81]	0.13	0.13	0.22
		Can f 6	0.39	[0.09; 0.68]	**0.01**	0.62	[0.03; 1.22]	0.04	**0.04**	0.12

PD_{20}	sIgE cat	Fel d 1	-0.94	[-2.03; 0.14]	0.09	0.06	[-1.07; 1.19]	0.92	0.92	0.92
		Fel d 4	-0.82	[-1.58; -0.07]	0.03	-1.05	[-3.39; 1.29]	0.38	0.38	0.63
	sIgE dog	Can f 1	-0.42	[-1.21; 0.37]	0.30	-1.26	[-2.92; 0.40]	0.14	0.14	0.35
		Can f 5	-0.58	[-1.39; 0.23]	0.16	-0.20	[-1.42; 1.03]	0.75	0.75	0.92
		Can f 6	-0.41	[-1.18; 0.36]	0.30	-1.81	[-3.71; 0.09]	0.06	0.06	0.32
Current asthma	sIgE cat	Fel d 1	3.08	[1.04; 9.13]	**0.04**	0.74	[0.24; 2.31]	0.60	0.60	0.85
		Fel d 4	2.38	[1.19; 4.77]	**0.01**	1.15	[0.29; 4.55]	0.85	0.85	0.85
	sIgE dog	Can f 1	2.41	[1.20; 4.87]	**0.01**	5.98	[1.15; 31.11]	**0.03**	**0.02**	0.09
		Can f 5	2.82	[1.38; 5.76]	**0.004**	1.74	[0.62; 4.85]	0.29	0.29	0.73
		Can f 6	3.21	[1.60; 6.43]	**0.001**	1.21	[0.32; 4.55]	0.78	0.78	0.85

Nested model comparison of pet sensitization measured by whole-extract sIgE and corresponding allergen components in relation to lung function, airway inflammation, airway hyperresponsiveness, and current asthma at 18 years. For each outcome, two models were compared: a base model including whole-extract sensitization (sIgE cat [e1] or sIgE dog [e5]) and covariates, and an extended model additionally including the relevant component (cat: Fel d 1 or Fel d 4; dog: Can f 1, Can f 5, or Can f 6). Linear regression was used for continuous outcomes and logistic regression for current asthma. Nested model comparisons were performed using an F-test for linear models and a likelihood ratio test (χ^2) for logistic models. P-values from the nested comparisons were adjusted for multiple testing using the Benjamini–Hochberg false discovery rate procedure within each outcome. Models were adjusted for HDM sensitization, current smoking, and current pet exposure (cat exposure in cat models; dog exposure in dog models). Estimates are presented as β for outcomes on the original scale, as fold-changes for outcomes modelled on the $\log_2$ scale (sRaw, FeNO, PD_{20}), and as odds ratios for current asthma.

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