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Original Article

Longitudinal trajectories of allergen sensitization in children with different transcriptome profiles from COCOA study

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Abbreviations:
AC, allergic conjunctivitis; AD, atopic dermatitis; AR, allergic rhinitis; *Der f*, *Dermatophagoides farina*; *Der p*, *Dermatophagoides pteronyssinus*; FA, food allergy; HDM, house dust mite; IgE, immunoglobulin E; SCORAD, SCORing Atopic Dermatitis

ABSTRACT

Background: Allergen sensitization trajectories are linked to diverse allergic disease phenotypes, but the underlying mechanisms remain unclear.
Methods: The study used skin prick test data obtained from children in the COCOA birth cohort (n = 549) at 3, 7, and 9 years of age. Sensitization to 13 aeroallergens, categorized into four groups (house dust mite [HDM], pollens, pets, and mold), was assessed and classified into trajectories using group-based multi-trajectory modeling. Blood transcriptome and cytokine analyses at age 7 were also performed.
Results: Four allergen sensitization trajectories were identified: “no sensitization” (42.8 %), “HDM only” (29.9 %), “pollen predominant” (6.0 %), and “multiple sensitization” (21.3 %). The “HDM only” and “pollen predominant” trajectories were associated with an increased risk of allergic rhinitis (AR) and allergic conjunctivitis. “Multiple sensitization” was associated with an increased risk of food allergy, atopic dermatitis, AR, and asthma during the first 9 years-of-age. IL-2 and IL-5 levels were elevated significantly in the “multiple sensitization” trajectory. Transcriptome analysis revealed that the “HDM only” trajectory was associated with B cell receptor responses, the “pollen predominant” trajectory was associated with MHC and T cell responses, and the “multiple sensitization” trajectory was associated with pathways related to barrier function, including IL-1 and tight junctions, while also sharing signaling pathways with both the “HDM only” and “pollen predominant” trajectories.
Conclusions: Four aeroallergen sensitization trajectories with distinct transcriptome profile during childhood were detected. “Multiple sensitization” trajectory is associated with an increased risk of allergic comorbidities, potentially mediated through barrier dysfunction.

Introduction

Atopy, a genetic predisposition to generating immunoglobulin E (IgE) antibodies specific for various environmental allergens or antigens, is closely associated with development of allergic

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diseases.[1] The patterns of allergen sensitization, defined as one or more positive responses to specific IgE antibody tests or skin prick tests (SPTs),[1] undergo dynamic changes, especially during childhood. Identification of distinct phenotypes of allergen sensitization based on the evolving patterns of sensitization in early life can provide valuable insights into the heterogeneity of allergic diseases.[2,3]

Some children with allergen sensitization remain asymptomatic; however, allergen sensitization is closely associated with the development, severity, and prognosis of various allergic diseases.[2,3] Notably, allergen sensitization has no single phenotype; rather, it encompasses diverse phenotypes that are classified based on factors such as the types of sensitized allergens, the timing of sensitization, and the degree of sensitization. The different phenotypes highlight the complexity of the relationship between allergen sensitization patterns and allergic diseases, particularly given the dynamic changes in allergen sensitization that occur throughout childhood. Understanding these patterns will provide critical insight into the development, progression, and prognosis of allergic diseases in children.[4]

Several studies have investigated the longitudinal patterns of allergen sensitization and their associations with allergic diseases in children[3,5]; however, these studies have limitations. Specifically, some focused on a limited range of allergens, thereby narrowing the scope of the findings,[5,6] whereas others examined only a restricted set of allergic disease outcomes in relation to allergen sensitization clusters, providing an incomplete picture.[3,7–9] Additionally, while many studies included follow-up periods during childhood, some were of relatively short duration, thereby limiting the ability to capture long-term patterns of allergen sensitization.[8,10] Most importantly, none have comprehensively explored the mechanisms underlying the diverse phenotypes of allergen sensitization in childhood. This leaves a significant gap in our understanding of the mechanisms underlying allergen sensitization patterns with allergic disease outcomes.

Here, we identified longitudinal allergen sensitization trajectories during childhood and investigated their association with allergic outcomes. Furthermore, we elucidated the mechanisms underlying these diverse allergen sensitization trajectories by analyzing blood transcriptome and cytokine profiles at 7 years of age.

Methods

Study population

This study was conducted as part of the Cohort for Childhood Origin of Asthma and allergic diseases (COCOA) study, a general population-based birth cohort in Seoul, Korea.[11] Pregnant women were recruited between November 19, 2007 and December 31, 2015 and mother–child pairs were followed-up from birth through clinic visits at 6 months, 1 year, and annually thereafter up to 12 years of age. Follow-ups included medical examinations by pediatric allergists and completion of a questionnaire at each visit. Among the total of 3102 participants in the COCOA birth cohort, 549 children with three consecutive SPT results at 3, 7, and 9 years of age were included in the present study. Baseline characteristics did not differ significantly between included and excluded children (Supplementary Table 1). Written informed consent was obtained from all parents and children. The cohort study protocol was approved by the institutional review boards of Asan Medical Center (2008–0616), Samsung Medical Center (2009-02-021), Severance Medical Center (4-2008-0588), CHA Medical Center (2010–010), and Seoul National University Hospital (H-1401-086-550).

Allergen sensitization

SPT results for 13 inhalant allergens (*Dermatophagoides pteronyssinus* [*Der p*], *Dermatophagoides farina* [*Der f*], alder, birch, oak, grasses, Japanese hop, mugwort, ragweed, cat, dog, *Alternaria alternata*, and *Aspergilus fumigatus*) at 3, 7, and 9 years were used for classification of allergen sensitization trajectories. Because group-based multi-trajectory modeling can include a maximum of 6–7 groups, the 13 allergens were categorized into four groups: house dust mite ([HDM], *Der p* and *Der f*), pollens (alder, birch, oak, grasses, Japanese hop, mugwort, and ragweed), pets (cats and dogs), and mold (*Alternaria alternata* and *Aspergilus fumigatus*). For trajectory modeling, each allergen group was considered 'positive' if at least one allergen within the group elicited a positive SPT response. Sensitization to food allergens was evaluated only for a limited number of allergens, and the prevalence of sensitization to food allergens (peanut, milk, egg white, and soybean) measured by SPTs was low in the present study (0.2–2.2 % at 3 years of age); therefore, food allergen sensitization results were not included in the classification of allergen sensitization trajectories. To maintain methodological consistency, allergen sensitization trajectories in this study were defined based on SPTs results for inhalant allergens, which were assessed uniformly at 3, 7, and 9 years of age. We selected the time points of 3, 7, and 9 years for trajectory analysis to capture the dynamic progression of allergen sensitization in childhood. Since allergen sensitization patterns evolve over time, assessing them at 2–3 years intervals provides a clearer understanding of long-term trajectories. A positive response to the SPT was defined as a mean wheal size of $\geq$3 mm for allergens, and a histamine level of 10 mg/mL, measured 15 min after administration.[2] Atopy was defined as a positive response in the SPTs.

Definitions of allergic outcomes

At each visit, participants and their parents completed a validated questionnaire on symptoms, treatment, and diagnosis of allergic diseases. These were reviewed in conjunction with comprehensive physical examinations conducted by pediatric allergists. A diagnosis of food allergy (FA) was based on a history of IgE-mediated symptoms following ingestion of specific food(s), supported by evidence of elevated levels of food allergen-specific IgE ($\geq$0.35 kU/L).[11,12] Atopic dermatitis (AD) was diagnosed by pediatric allergists based on the criteria outlined by Hanifin and Rajka.[11,13] Allergic rhinitis (AR) was defined as the presence of two or more symptoms (i.e., watery rhinorrhea, nasal itching, sneezing, and nasal obstruction), confirmed by physical examination conducted by pediatric allergy specialists.[14] Asthma was diagnosed if participants reported two or more episodes of wheezing, two or more visits to the doctor due to asthma or wheezing, at least one hospitalization for asthma or wheezing, or the use of asthma medications within the past 12 months.[11,12]

Measurement of eosinophil (%), total serum IgE levels and cytokines in blood

Blood eosinophil percentage and total serum IgE levels were measured at birth and at ages 1, 3, 7, and 9 years. Cytokine levels in plasma were analyzed at 7 years of age by a multiplex technique using Human High Sensitivity Cytokine Kits (Magnetic Luminex® Performance Assay, R&D Systems, Minneapolis, MN, USA) (see the Online Repository for more details).

Blood transcriptome analysis

We selected the 7-year time point for omics analysis because allergen sensitization patterns become more stable at this age, and it marks the beginning of school age, a period when asthma can be more clearly defined. Peripheral blood samples obtained at 7 years-of-age were collected in PAXgene tubes (PreAnalytiX, Qiagen/BD, Hilden, Germany). Among the subjects, blood samples with typical characteristics in each trajectory group were selected to reduce bias, as blood samples stored in the PAXgene tubes were limited. Transcriptome analysis of samples from 58 subjects (18 "no sensitization", 16 "HDM only sensitization", eight "pollen predominant sensitization", and 16 "multiple sensitization") was performed using the GeneChip® Human Gene 2.0 ST Array (Affymetrix, Santa Clara, CA). Genes showing a change in expression (a |fold change| (|FC|) $\geq$ 1.5 and a *t*-test *P*-value <0.05) were considered to be differentially expressed genes (DEGs; see the Online Repository for more details). False discovery rate was controlled by adjusting *P*-value using Benjamini-Hochberg algorithm implemented in the R software (version 4.1.2, R Foundation for Statistical Computing, Vienna, Austria).

Statistical analysis

The trajectories of allergen sensitization patterns during childhood were examined using group-based multi-trajectory modeling, which is a statistical approach that identifies distinct subgroups showing different longitudinal patterns over time.[15] Allergen sensitization was modeled as a categorical measure (no sensitization *vs*. sensitization) based on the results of the SPTs. The age at which the SPTs were performed was treated as the primary time factor. Group-based multi-trajectory modeling was conducted using data from participants who had complete sensitization data at all three time points. The trajectory groups were identified through data-driven analysis using an unsupervised modeling framework. The optimal number of trajectory groups (n = 4) was determined based on model fit, guided by the Akaike Information Criterion (−1724.14) and the Bayesian Information Criterion (−1793.07). Each individual was probabilistically assigned to one of the four groups, with posterior probabilities exceeding 0.7 in all cases. Models with 2–6 groups were evaluated, and the final model was selected using the PROC TRAJ procedure in SAS version 9.4. Group labels ("no sensitization", "HDM only", "pollen predominant", and "multiple sensitization") were assigned post hoc for clarity and interpretability after data-driven identification of trajectory groups. The characteristics of individuals within each trajectory group were analyzed using appropriate statistical tests, e.g., Chi-square tests for categorical variables, and ANOVA or Kruskal–Wallis tests for continuous variables. The associations between each trajectory and allergic diseases were investigated using logistic regression analysis, referenced to the "no sensitization" group. Logistic regression analysis was adjusted for sex, family history of allergic diseases, maternal educational levels, and breastfeeding in the first 6 months.

Table 1
Baseline characteristics of the study population according to atopic sensitization trajectories.

N (%) or mean ± SD	Overall	Trajectory 1 (No sensitization)	Trajectory 2 (HDM only)	Trajectory 3 (Pollen predominant)	Trajectory 4 (Multiple sensitization)	*P*-value
Number (%)	549	235 (42.81 %)	164 (29.87 %)	33 (6.01 %)	117 (21.31 %)	NA
Sex, male	282 (51.4 %)	116/235 (49.4 %)	79/164 (48.2 %)	20/33 (60.6 %)	67/117 (57.3 %)	0.283
Cesarean section delivery	169 (31.7 %)	71/228 (31.1 %)	51/160 (31.9 %)	11/32 (34.4 %)	36/113 (31.9 %)	0.986
Gestational age, weeks	39.1 ± 1.2	39.2 ± 1.1	39.1 ± 1.2	38.7 ± 1.3	39.2 ± 1.2	0.379
Maternal education levels						0.180
≤ high school	29/549 (5.3 %)	16/235 (6.8 %)	6/164 (3.7 %)	2/33 (6.1 %)	5/117 (4.3 %)	
University	400/549 (72.9 %)	175/235 (74.5 %)	116/164 (70.7 %)	28/33 (84.9 %)	81/117 (69.2 %)	
≥ Graduate school	120/549 (21.9 %)	44/235 (18.7 %)	42/164 (25.6 %)	3/33 (9.1 %)	31/117 (26.5 %)	
Breastfeeding for the first 6 months	395/546 (72.3 %)	167/235 (71.1 %)	121/164 (73.8 %)	23/31 (74.2 %)	84/116 (72.4 %)	0.937
Maternal exposure to ETS during pregnancy	344/536 (64.2 %)	138/228 (60.5 %)	109/163 (66.9 %)	18/32 (56.3 %)	79/113 (69.9 %)	0.228
Family history of allergic diseases	267/549 (48.6 %)	116/235 (49.4 %)	78/164 (47.6 %)	13/33 (39.4 %)	60/117 (51.3 %)	0.664
Maternal AD	43/547 (7.9 %)	24/234 (10.3 %)	10/163 (6.1 %)	1/33 (3.0 %)	8/117 (6.8 %)	0.289
Maternal AR	157/547 (28.7 %)	65/234 (27.8 %)	46/163 (28.2 %)	9/33 (27.3 %)	37/117 (31.6 %)	0.888
Maternal asthma	16/547 (2.9 %)	7/234 (3.0 %)	5/163 (3.1 %)	0/33 (0.0 %)	4/117 (3.4 %)	0.775
Paternal AD	34/548 (6.2 %)	13/235 (5.5 %)	11/164 (6.7 %)	1/33 (3.0 %)	9/116 (7.8 %)	0.728
Paternal AR	114/547 (20.8 %)	47/234 (20.1 %)	34/164 (20.7 %)	6/33 (18.2 %)	27/116 (23.3 %)	0.887
Paternal asthma	16/547 (2.9 %)	4/234 (1.7 %)	7/164 (4.3 %)	1/33 (3.0 %)	4/116 (3.4 %)	0.499
Parental atopy on skin prick tests, yes	309/540 (57.2 %)	116/230 (50.4 %)	91/160 (56.9 %)	22/33 (66.7 %)	80/117 (68.4 %)	**0.009**
Birth seasons						0.056
Spring	126/547 (23.0 %)	61/235 (26 %)	36/164 (22 %)	7/33 (21.2 %)	22/117 (18.8 %)	
Summer	130/549 (23.7 %)	54/235 (23 %)	48/164 (29.3 %)	3/33 (9.1 %)	25/117 (21.4 %)	
Autumn	139/549 (25.3 %)	63/235 (26.8 %)	41/164 (25 %)	9/33 (27.3 %)	26/117 (22.2 %)	
Winter	154/549 (28.1 %)	57/235 (24.3 %)	39/164 (23.8 %)	14/33 (42.4 %)	44/117 (37.6 %)	

AD, atopic dermatitis; AR, allergic rhinitis; N, number; ETS, environmental Tobacco Smoke; NA, not applicable; SD, standard deviation.

The Enrichr program (https://maayanlab.cloud/Enrichr/), which evaluates gene set enrichment and integrates the context of known biological processes, including the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO), was used to investigate the underlying mechanisms. All statistical analyses were performed using R software (version 4.1.2), except for group-based trajectory modeling.

Additional methods: Additional Methods: See the supplementary files.

Results

Characteristics of the study population

This study included 549 children, of whom 282 (51.4 %) were boys and 267 (48.6 %) were girls. A total of 267 (48.6 %) had a parental history of allergic diseases (Table 1). The four-group model revealed the best fit based on Bayesian information criteria and Akaike information criteria. The rate of parental atopy was highest in "multiple sensitization" trajectory and lowest in "no sensitization" trajectory. Flowchart of the research design and subject inclusion is shown in Supplementary Figure 1.

Allergen sensitization status in each trajectory and total population

Supplementary Table 2 summarizes the prevalence of sensitization to specific allergens within each allergen sensitization trajectory and in the total population, at 3, 7, and 9 years of age. Across all ages, sensitization to *Der p* and *Der f* was most common in the total population, with sensitization rates increasing significantly with age (*Der p*: 11.8 % at 3 years and 40.8 % at 9 years; *Der f*: 12.8 % at 3 years and 46.8 % at 9 years). Among pollen allergens, sensitization to tree pollens such as alder, birch, and oak at 7 and 9 years of age was more common than sensitization to grass pollens. Sensitization to pet allergens (cats and dogs) also increased with age. Sensitization to cat allergens rose from 1.5 % at 3 years to 8.7 % at 9 years, with the "multiple sensitization" trajectory showing the highest sensitization rates (35.0 % at 9 years of age). Similarly, sensitization to dog allergens increased from 1.1 % at 3 years to 4.7 % at 9 years, with the "multiple sensitization" trajectory showing the highest prevalence (17.9 % at 9 years of age).

Allergen sensitization trajectories

Trajectory 1 ("no sensitization", n = 235/549, 42.8 %) demonstrated minimal allergen sensitization throughout childhood (Table 1 and Fig. 1). Trajectory 2 ("HDM only", n = 164/549, 29.9 %) exhibited increasing sensitization to HDM with age, with little to no sensitization to other allergen groups. Trajectory 3 ("pollen predominant", n = 33/549, 6.0 %) predominantly involved sensitization to pollens, along with mild sensitization to pets and molds, but negligible sensitization to HDM. Trajectory 4 ("multiple sensitization", n = 117/549, 21.3 %) was characterized by increasing multi-sensitization to HDM, pollens, and pets with age, as well as, albeit weaker, sensitization to molds.

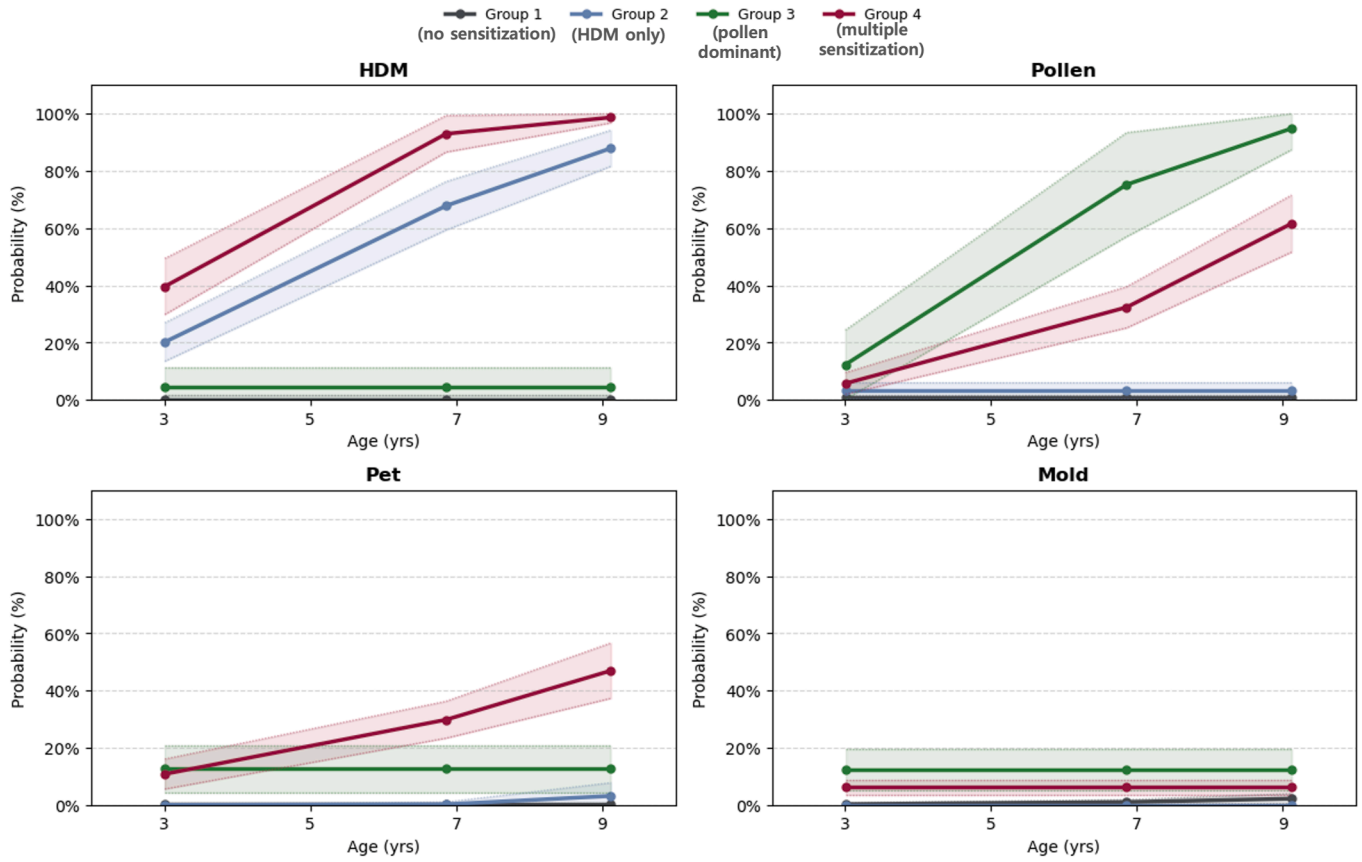

Fig. 1. Longitudinal allergen sensitization trajectories by allergen groups. Probability of sensitization to each allergen group (house dust mite [HDM], pollens, pets, and mold) at 3, 7, and 9 years of age according to the four identified allergen sensitization trajectories: "no sensitization" (Group 1, 42.8 %), "HDM only" (Group 2, 29.9 %), "pollen predominant" (Group 3, 6.0 %), and "multiple sensitization" (Group 4, 21.3 %). Solid lines represent estimated probabilities, and shaded areas represent 95 % confidence intervals. HDM, house dust mite.

Table 2
Associations between atopic sensitization clusters and allergic disease outcomes.

Ages	Outcomes	Trajectory 1 (No sensitization)	Trajectory 2 (HDM only)	Trajectory 3 (Pollen predominant)	Trajectory 4 (Multiple sensitization)
0–1 yrs	FA diagnosis ever	1 [ref]	1.44 (0.56–3.68)	0.80 (0.10–6.56)	**2.46 (0.99–6.08)**
	FA diagnosis with sensitization to egg white or milk at age 1	1 [ref]	**2.30 (1.23–4.31)**	2.13 (0.72–6.24)	**2.70 (1.39–5.26)**
	AD diagnosis ever	1 [ref]	0.96 (0.59–1.59)	1.53 (0.65–3.57)	**2.12 (1.29–3.49)**
	AR diagnosis ever	1 [ref]	2.89 (0.26–32.43)	8.74 (0.52–145.99)	1.87 (0.11–30.61)
	AR symptom ever	1 [ref]	1.23 (0.74–2.07)	0.97 (0.35–2.72)	1.50 (0.86–2.61)
	Wheeze or asthma diagnosis ever	1 [ref]	1.34 (0.42–4.27)	1.26 (0.14–10.99)	0.85 (0.21–3.51)
0–3 yrs	FA diagnosis ever	1 [ref]	**2.5 (1.22–5.13)**	1.13 (0.24–5.30)	**3.07 (1.45–6.50)**
	AD diagnosis ever	1 [ref]	1.11 (0.71–1.73)	1.26 (0.56–2.84)	**2.51 (1.57–4.00)**
	AR diagnosis ever	1 [ref]	0.79 (0.39–1.63)	1.56 (0.49–4.92)	1.37 (0.68–2.76)
	Wheeze or asthma diagnosis ever	1 [ref]	1.18 (0.60–2.34)	1.81 (0.62–5.29)	1.17 (0.56–2.45)
0–6 yrs	FA diagnosis ever	1 [ref]	**1.85 (0.97–3.51)**	1.25 (0.34–4.53)	**3.29 (1.73–6.26)**
	AD diagnosis ever	1 [ref]	1.05 (0.69–1.61)	0.91 (0.41–2.04)	**2.47 (1.56–3.91)**
	AR diagnosis ever	1 [ref]	1.22 (0.81–1.84)	1.38 (0.64–2.94)	**2.93 (1.84–4.68)**
	Wheeze or asthma diagnosis ever	1 [ref]	1.71 (0.94–3.09)	1.60 (0.55–4.61)	1.16 (0.58–2.32)
0–9 yrs	FA diagnosis ever	1 [ref]	1.33 (0.74–2.41)	2.04 (0.75–5.55)	**2.79 (1.55–5.02)**
	AD diagnosis ever	1 [ref]	1.03 (0.68–1.55)	0.86 (0.39–1.89)	**2.25 (1.42–3.55)**
	AR diagnosis ever	1 [ref]	**2.89 (1.85–4.51)**	**3.92 (1.54–10.00)**	**7.99 (4.14–15.41)**
	Wheeze or asthma diagnosis ever	1 [ref]	1.62 (0.94–2.80)	1.52 (0.57–4.06)	1.36 (0.74–2.50)
7–12 years	Asthma diagnosis ever between 4 and 7 years with Provocholine® $PC_{20} \leq 4$ mg/dL at 7 or 9 years of age	1 [ref]	1.45 (0.91–2.31)	0.76 (0.28–2.10)	**1.73 (1.04–2.87)**
	Asthma diagnosis	1 [ref]	1.46 (0.63–3.38)	1.95 (0.51–7.48)	**3.11 (1.43–6.78)**
	AD diagnosis	1 [ref]	1.34 (0.83–2.16)	0.59 (0.20–1.79)	**2.08 (1.25–3.44)**
	FA diagnosis	1 [ref]	0.73 (0.32–1.69)	1.52 (0.41–5.65)	**2.77 (1.38–5.57)**
	Allergic conjunctivitis diagnosis	1 [ref]	**3.41 (2.03–5.73)**	**7.35 (3.23–16.72)**	**8.26 (4.79–14.25)**
	Oral allergy syndrome symptom	1 [ref]	**3.12 (1.14–8.54)**	**6.20 (1.62–23.71)**	**8.86 (3.41–23.01)**

aOR, adjusted odds ratio; AD, atopic dermatitis; AR, allergic rhinitis; CI, confidence interval; FA, food allergy; yrs, years.
Bold data are statistically significant ($P < 0.05$).
Adjusted for maternal education, infant's sex, parental history of allergy, and breastfeeding within the first 6 months.

Associations of allergen sensitization trajectories with clinical outcomes during the childhood

The association between longitudinal allergen sensitization trajectories and allergic disease outcomes is summarized in Table 2. The "HDM only" and "multiple sensitization" trajectories were significantly associated with a diagnosis of FA during the first year of life, particularly in cases of sensitization to egg white or milk at age 1, with stronger associations observed in the "multiple sensitization" trajectory (adjusted odds ratio [aOR]: 2.30; 95 % CI: 1.23–4.31 for "HDM only" and aOR: 2.70; 95 % CI: 1.39–5.26 for "multiple sensitization"). During the first 3 years of life, the "multiple sensitization" trajectory showed the strongest association with an FA diagnosis (aOR: 3.07; 95 % CI: 1.45–6.50), followed by the "HDM only" trajectory (aOR: 2.50; 95 % CI: 1.22–5.13). The "multiple sensitization" trajectory was also associated with a diagnosis of AD during the first 3 years of life (aOR: 2.51; 95 % CI: 1.57–4.00).

By the age of 6 years, the "multiple sensitization" trajectory continued to demonstrate a significant association with multiple allergic outcomes, including a diagnosis of FA (aOR: 3.29; 95 % CI: 1.73–6.26), AD (aOR: 2.47; 95 % CI: 1.56–3.91), or AR (aOR: 2.93; 95 % CI: 1.84–4.68). Additionally, at 9 years of age, the "multiple sensitization" trajectory remained significantly associated with an FA diagnosis (aOR: 2.79; 95 % CI: 1.55–5.02) and an AD diagnosis (aOR: 2.25; 95 % CI: 1.42–3.55). An AR diagnosis was associated with multiple trajectories, including "HDM only" (aOR: 2.89; 95 % CI: 1.85–4.51), "pollen predominant" (aOR: 3.92; 95 % CI: 1.54–10.00), and "multiple sensitization" (aOR: 7.99; 95 % CI: 4.14–15.41).

Total serum IgE and cytokine levels across four allergen sensitization trajectories

There were significant differences between the four allergen sensitization trajectories with respect to immune profiles obtained from cord blood samples, and paternal total serum IgE levels (Table 3). Total serum IgE levels at ages 1, 3, 7, and 9 were consistently highest in the "multiple sensitization" trajectory ($P < 0.001$, except those at 3 years of age). Paternal total serum IgE levels were significantly higher in the "multiple sensitization" trajectory (375.63 ± 772.5 kU/L; $P < 0.001$) than in the other trajectories. Cord blood cell proliferation responses to phytohemagglutinin were also notably higher in the "multiple sensitization" trajectory (4.25 ± 1.17; $P = 0.003$). Regarding allergen-specific IgE levels, the "multiple sensitization" trajectory had the highest levels of egg white- and milk-specific IgE at age 1 ($P < 0.001$ and $P = 0.030$, respectively). By 9 years of age, blood eosinophils (%) were highest in the "multiple sensitization" trajectory. There were significant differences in the concentration of serum IL-2 at 7 years of age across the trajectories, with the highest levels being observed in the "multiple sensitization" trajectory (Fig. 2). The highest levels of serum IL-5 were also observed in the "multiple sensitization" trajectory, followed by the "HDM only", "pollen predominant", and "no sensitization" trajectories.

Table 3
Comparison of biomarkers across atopy sensitization trajectories.

Mean ± SD	Trajectory 1 (No sensitization)		Trajectory 2 (HDM only)		Trajectory 3 (Pollen predominant)		Trajectory 4 (Multiple sensitization)		*P* value
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	
Log transformed cord blood IFN-γ (pg/mL)	91	5.36 ± 1.32	64	5.21 ± 1.77	10	5.40 ± 1.26	37	5.48 ± 1.44	0.690
Log transformed cord blood IL-13 (pg/mL)	97	7.20 ± 1.05	70	7.06 ± 1.17	12	7.02 ± 0.72	38	7.01 ± 0.95	0.310
Log transformed cord blood OVA/media	94	0.39 ± 0.69	66	0.50 ± 0.67	10	0.35 ± 0.62	39	0.57 ± 0.50	0.190
Maternal total serum IgE level (kU/L)	203	126.27 ± 386.82	140	119.97 ± 428.13	26	241.68 ± 710.42	102	88.92 ± 178.46	0.650
Maternal blood eosinophil (%)	186	1.14 ± 1.19	135	1.01 ± 1.16	30	1.17 ± 0.89	90	1.09 ± 1.11	0.800
Paternal total serum IgE (kU/L)	178	130.36 ± 243.99	122	266.72 ± 540.92	28	240.98 ± 743.77	93	375.63 ± 772.5	**< 0.001**
Paternal blood eosinophil (%)	190	3.14 ± 2.29	130	3.92 ± 3.14	30	4.96 ± 6.74	94	3.63 ± 3.79	0.100
Cord blood total serum IgE (kU/L)	68	1.96 ± 0.37	47	1.92 ± 0.17	7	2.04 ± 0.36	41	2.16 ± 1.17	0.110
Cord blood eosinophil (%)	109	3.01 ± 1.80	80	3.51 ± 2.03	19	4.59 ± 4.08	64	3.71 ± 2.30	**0.020**
Total serum IgE at age 1 yr (kU/L)	203	37.31 ± 64.67	134	47.37 ± 61.18	25	45.12 ± 72.19	99	79.98 ± 141.75	**< 0.001**
Blood eosinophil (%) at age 1 yr	209	2.79 ± 1.78	142	2.78 ± 1.80	27	3.37 ± 2.24	97	2.88 ± 1.68	0.460
Egg white specific IgE at age 1 yr (kU/L)	203	0.14 ± 0.40	134	0.89 ± 2.65	25	0.95 ± 2.04	99	3.53 ± 11.67	**< 0.001**
Milk specific IgE at age 1 yr (kU/L)	203	0.28 ± 1.03	134	0.56 ± 1.58	25	0.21 ± 0.29	99	0.82 ± 3.12	**0.030**
Total serum IgE at age 3 yrs (kU/L)	201	107.21 ± 385.74	143	133.94 ± 262.4	29	109.24 ± 174.29	103	155.3 ± 259.27	0.240
Blood eosinophil (%) at age 3 yrs	216	2.98 ± 2.11	153	3.42 ± 2.84	32	3.55 ± 2.36	107	3.74 ± 2.25	**0.006**
Egg white specific IgE at age 3 yrs (kU/L)	201	0.22 ± 0.49	143	0.96 ± 8.35	29	0.27 ± 0.38	103	0.58 ± 1.39	0.590
Milk specific IgE at age 3 yrs (kU/L)	201	0.25 ± 0.43	143	0.38 ± 0.72	29	0.21 ± 0.28	103	0.37 ± 0.75	0.140
Der f specific IgE at age 3 yrs (kU/L)	197	2.72 ± 10.96	140	3.85 ± 14.83	28	4.22 ± 17.63	100	5.79 ± 18.22	0.080
Total serum IgE at age 7 yrs (kU/L)	217	116.32 ± 389.41	149	225.56 ± 285.61	33	178.08 ± 275.03	106	438.95 ± 582.61	**< 0.001**
Blood eosinophil (%) at age 7 yrs	213	2.81 ± 2.15	150	3.9 ± 3.06	31	3.81 ± 4.13	104	5.45 ± 3.3	**< 0.001**
Egg white specific IgE at age 7 yrs (kU/L)	58	0.09 ± 0.14	43	0.21 ± 0.25	7	0.27 ± 0.18	36	0.66 ± 1.56	**0.001**
Milk specific IgE at age 7 yrs (kU/L)	58	0.11 ± 0.16	43	0.23 ± 0.31	7	0.22 ± 0.32	36	0.2 ± 0.29	0.080
Der f specific IgE at age 7 yrs (kU/L)	217	2.04 ± 11.12	149	19.71 ± 29.62	33	0.85 ± 4.2	106	43.73 ± 40.76	**< 0.001**
Birch specific IgE at age 7 yrs (kU/L)	216	0.02 ± 0.12	148	0.12 ± 0.6	33	8.32 ± 21.26	104	4.82 ± 14.05	**< 0.001**
Alternaria specific IgE at age 7 yrs (kU/L)	216	0.80 ± 4.96	148	0.11 ± 1.10	33	4.02 ± 8.56	104	0.85 ± 3.59	0.300
Total serum IgE at age 9 yrs (kU/L)	204	132.23 ± 369.95	146	247.72 ± 314.05	28	253.05 ± 376.46	106	528.14 ± 632.22	**< 0.001**
Blood eosinophil (%) at age 9 yrs (kU/L)	223	2.58 ± 2.57	159	3.67 ± 2.41	30	3.51 ± 2.3	116	4.76 ± 2.58	**< 0.001**
Der f specific IgE at age 9 yrs (kU/L)	202	0.90 ± 6.84	141	31.61 ± 54.21	28	2.92 ± 7.81	101	51.08 ± 37.33	**< 0.001**
Birch specific IgE at age 9 yrs (kU/L)	202	0.11 ± 0.88	143	0.61 ± 4.53	26	10.76 ± 21.72	106	8.82 ± 18.52	**< 0.001**
Alternaria specific IgE at age 9 yrs (kU/L)	202	0.66 ± 4.15	141	1.36 ± 14.71	25	5.16 ± 14.18	105	2.86 ± 9.7	**0.04**

Bold data are statistically significant ($P < 0.05$).
Der f, *Dermatophagoides farinae*; IgE immunoglobulin E; IFN-γ, interferon-γ; IL-13, interlukin-13; OVA, ovalbumin; SD, standard deviation.

Potential mechanisms by transcriptomic profiles and network analyses

Global gene expression profiling of blood samples (Supplementary Table 3) revealed that differential expression of several genes important for allergic and immune responses (i.e., *CFD*, *CLC*, *FCRL5*, and *TYROBP*) overlapped among the "HDM only", "pollen predominant", and "multiple sensitization" trajectories (Supplementary Table 4). Comparison of gene enrichment analysis data from the "no sensitization" trajectory with those from each of the other three trajectories (individually), revealed differential expression of genes related to the following pathways and biological processes (Supplementary Tables 5–7 and Fig. 3): (1) B cell receptor signaling ($P = 2.85 \times 10^{-5}$) in the "HDM only" trajectory; (2) peptide antigen assembly with MHC class II protein complex ($P = 0.01$) and regulation of T cell activation ($P = 0.04$) in the "pollen predominant" trajectory; and (3) regulation of TGF-β1 production ($P = 0.009$) in the "multiple sensitization" trajectory. The "multiple sensitization" trajectory also shared signaling processes with the "HDM only" and "pollen predominant" sensitization trajectories (i.e., B cell proliferation/activation and T cell regulation).

Direct comparison of DEGs in the "HDM only" and "pollen predominant" trajectories (Supplementary Table 8) revealed that both were enriched in C-type lectin receptor signaling pathways ($P = 0.005$, Supplementary Table 9), which recognize allergens derived from HDMs and plays a critical role in immune responses. Further direct comparison of the "multiple sensitization" and the combined "HDM only" and "pollen predominant" trajectories (Supplementary Table 8) revealed notable enrichment of pathways related to IL-1 regulation ($P = 0.008$, Supplementary Table 10) and tight junctions ($P = 0.03$).

Public DB-based interaction network analysis of DEGs using the GeneMANIA program indicated potential cored interaction networks for each trajectory ("HDM only", "pollen predominant", and "multiple sensitization"); these included important genes involved in allergic responses and sensitization (i.e., *CLC*, *FCRL5*, *JCHAIN*, and *TYROBP* in the "HDM only" trajectory; *HLA-DQA1* and *FCRL5* in the "pollen predominant" trajectory; and *CLC*, *RNASE2*, *ALAS2*, and *TYROBP* in the "multiple sensitization" trajectory; Fig. 4A–4C), even though there was relatively distinguishable clustering among these three trajectories (Fig. 4D). In addition, differential expression of these genes was trajectory-dependent (Supplementary Fig. 2). Further DEG-based network analysis using Cytoscape software revealed that immunoglobulin genes involved in biological pathways in the "HDM only" and "pollen predominant" trajectories formed a cored interaction network (Supplementary Fig. 3).

Correlation between network-cored DEGs and clinical parameters

Additional correlation analyses between network-cored gene expression and clinical parameters showed significant differences among three trajectories (Fig. 5A). In particular, *Der p*-specific IgE level at 3 years of age was negatively correlated with *CFD* expression in "HDM only" ($r = -0.582$, $P = 0.037$, Fig. 5B), but inversely correlated in "pollen predominant" trajectory; birch-specific IgE level at 9 years of age showed highest negative correlation with *IGLL5* expression in "pollen predominant" trajectory ($r = -0.865$, $P = 0.006$, Fig. 5C); total serum IgE level at 3 years of

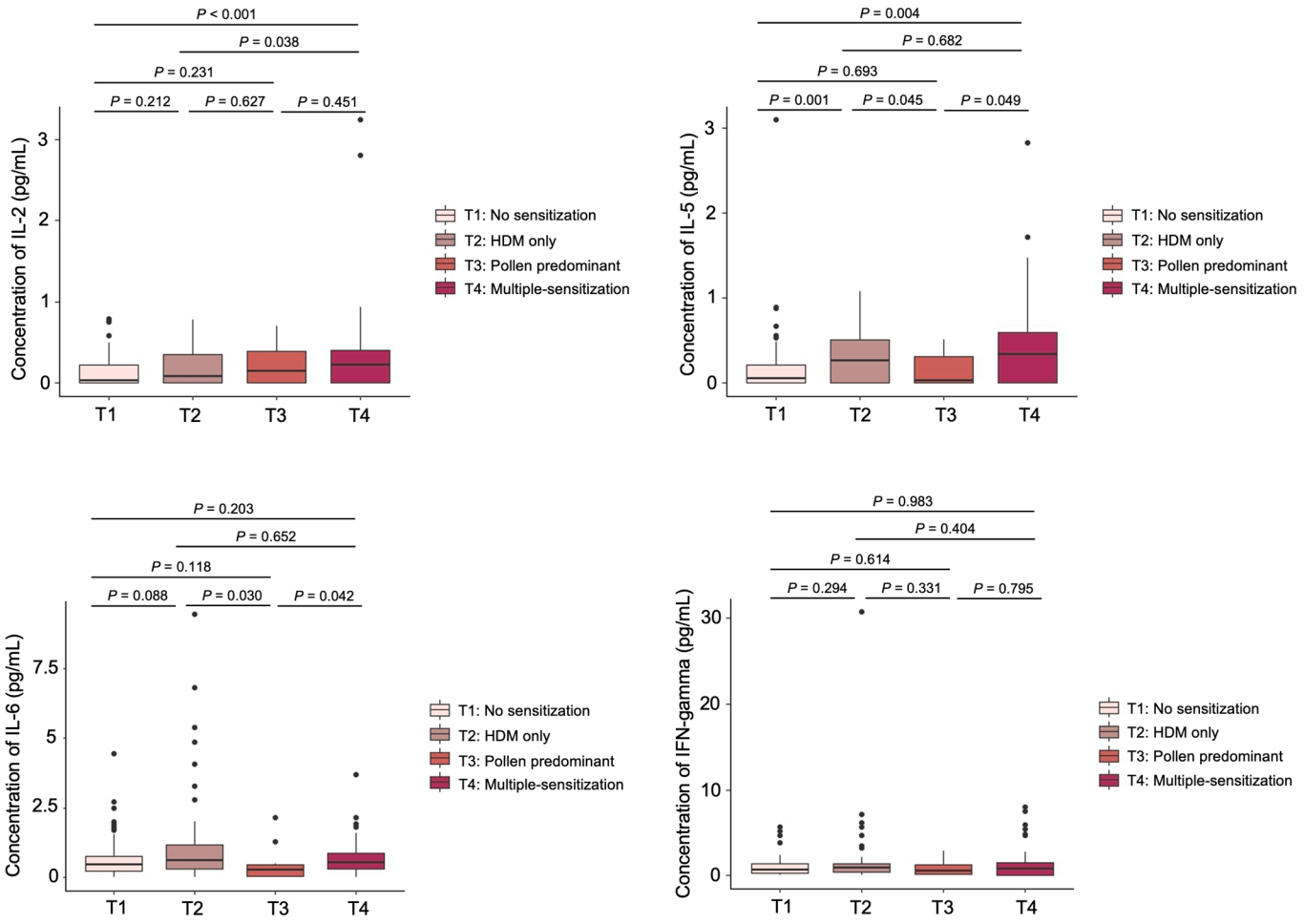

Fig. 2. Comparisons of blood cytokine levels. Comparisons of blood cytokine levels measured at 7 years of age across the four allergen sensitization trajectories.

age was negatively correlated with *TYROBP* expression in "multiple sensitization" trajectory ($r = -0.811$, $P = 0.004$, Fig. 5D), but not correlated in "HDM only" and inversely correlated in "pollen predominant" trajectory.

Discussion

We identified four distinct aeroallergen sensitization trajectories occurring during childhood: "no sensitization", "HDM only", "pollen predominant", and "multiple sensitization". Each trajectory was associated with different allergic outcomes throughout childhood. Notably, the "multiple sensitization" trajectory showed the strongest association with diverse allergic diseases, including AD, FA, AR, and asthma. In addition, IL-5 and IL-2 levels were elevated significantly in the "multiple sensitization" trajectory. The transcriptome data with cytokine profiles presented herein provide new insights into the mechanisms underlying these diverse aeroallergen sensitization trajectories. Specifically, the "HDM only" trajectory was associated with B cell receptor responses, the "pollen predominant" trajectory was associated with MHC and T cell responses, and the "multiple sensitization" trajectory not only shared signaling pathways with the "HDM only" and "pollen predominant" trajectories but also showed associations with pathways related to barrier function. These findings offer a fundamentally different framework for understanding the heterogeneous aeroallergen sensitization trajectories during childhood and their differential clinical outcomes, as well as their characteristic mechanisms, paving the way for tailored prevention and therapeutic strategies for childhood allergen sensitization trajectories with the subsequent allergic outcomes.

There have been several studies investigating longitudinal allergen sensitization trajectories during childhood, each providing insights into the relationship between sensitization patterns and allergic diseases (Supplementary Table 11).[3,5–10,16,17] These studies generally highlight the dynamic nature of allergen sensitization patterns over time and their associations with specific allergic outcomes. A birth cohort study in Singapore identified three sensitization trajectories, emphasizing the role of early sensitization in predicting wheeze, AD, and AR.[5] A Japanese birth cohort reported age-specific sensitization patterns using component-resolved diagnostics, showing that multiple allergen sensitizations at age 9 were associated with childhood asthma, rhinitis, and AD.[9] Another study analyzing children from Finnish and Estonian cohorts reported similar latent classes of allergen sensitization in children, despite differences in environmental and socioeconomic factors[10]; however, these studies focused on a limited set of allergens or short follow-up periods, restricting their generalizability and ability to capture the full spectrum of allergen sensitization trajectories. By contrast, a strength of the current study is inclusion of a broader range of allergens (i.e., 13 common inhalant allergens), and extension of the follow-up period to 9 years of age for aeroallergen sensitization trajectories and 12 years

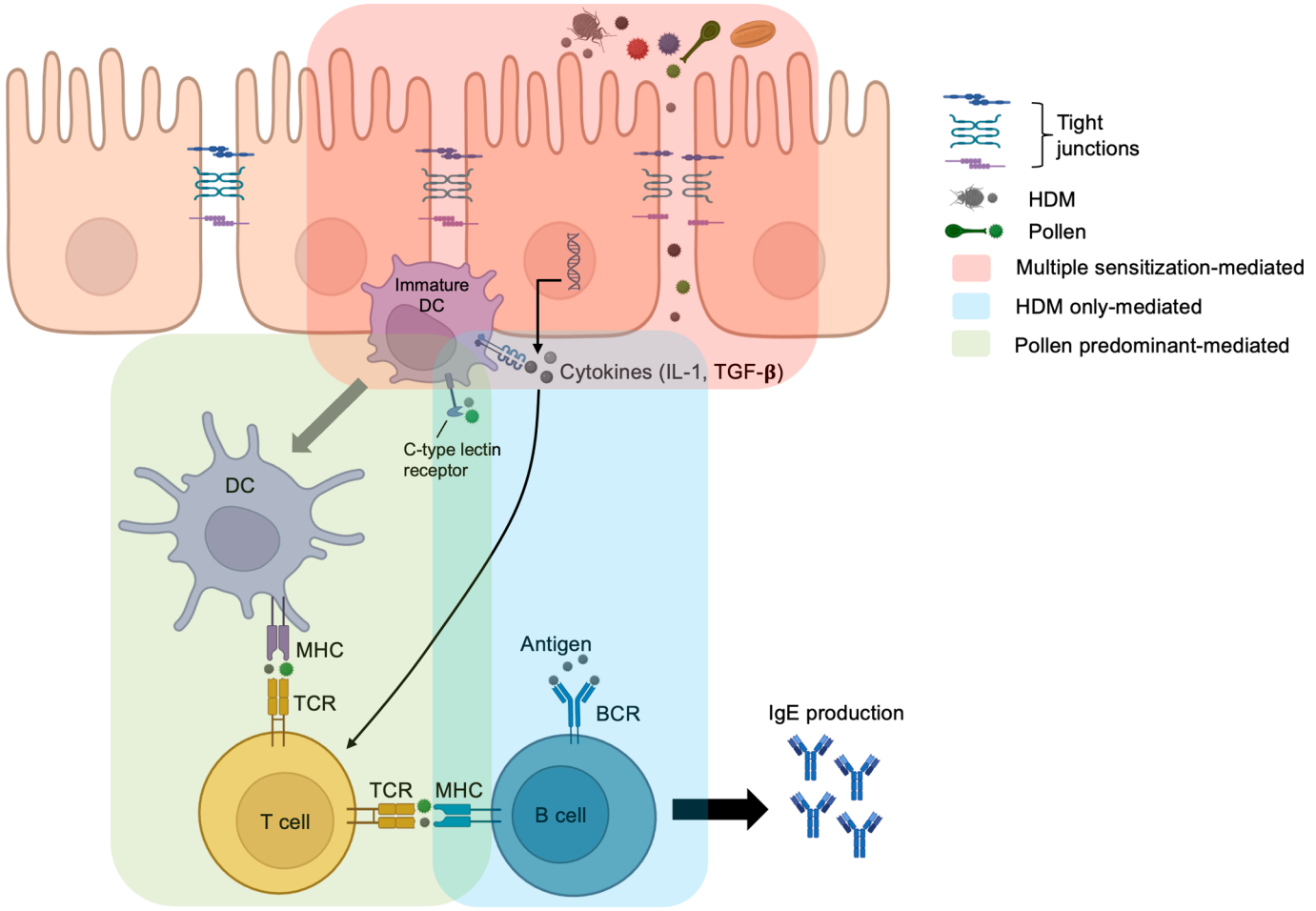

Fig. 3. Schematic potential mechanisms of the allergen sensitization trajectories. Briefly, B cell receptor signaling is enriched and may therefore play an important role in the "HDM only" trajectory, whereas the assembly of peptide antigens with MHC class II molecules and subsequent T cell regulation are enriched in the 'pollen predominant' trajectory. However, C-type lectin receptor signaling may have a differential role between "HDM only" and "pollen predominant" trajectories. However, C-type lectin receptor signaling may play different roles in the "HDM only" and "pollen predominant" trajectories. Tight junctions and cytokines, including TGF-β1 and IL-1, may be more important in the "multiple sensitization" trajectory than in the "HDM only" and "pollen predominant" trajectories. Figure was created with BioRender.com.

of age for allergic disease outcomes. Furthermore, we provide mechanistic insight into shared and distinct immunological pathways among the four aeroallergen sensitization trajectories. In addition, by employing group-based multi-trajectory modeling, our study identifies multiple sensitization trajectories concurrently, offering a dynamic understanding of how sensitization evolves over time. The use of a general population-based birth cohort means that the data are more generalizable than those reported by prior studies, which were often limited to high-risk cohorts.

Allergen sensitization reflects an individual's susceptibility to environmental factors; however, we found no significant differences between the majority of environmental factors to which individuals were exposed during the prenatal period and early childhood; the exceptions were pet ownership during pregnancy and exposure to environmental tobacco smoke during the first 6 months of life (Supplementary Table 12). A previous study conducted across two regions with profoundly different environmental exposures reported similar patterns of allergen sensitization clusters during early childhood.[10] These findings suggest that the comprehensive and complex interactions between genetic predisposition to barrier dysfunction and immune-related mechanisms might play critical roles in shaping allergen sensitization trajectories during childhood.

A high total serum IgE level may be associated with specific IgE levels, although total serum IgE levels are not equivalent to the sum of the specific IgE levels. In our study, total serum IgE levels at ages 1, 3, and 7 years were highest in the 'multiple sensitization' trajectory, followed by the "HDM only", "pollen predominant", and "no sensitization" trajectories. IL-5 levels showed a similar pattern, suggesting a strong association between the "multiple sensitization" trajectory and the strongest IgE-mediated responses, characterized by increased activation and survival of eosinophils. Elevated total serum IgE levels in the "HDM only" trajectory may be linked to activation of B cell receptors, which are crucial for IgE production, combined with eosinophilic inflammation. This mechanism could also explain the increased risk of FA in this trajectory, with sensitization to food allergens at age 1 and FA persisting until age 3.

By contrast, the "pollen predominant" trajectory did not show a significant association with allergic outcomes in early life. Notably, despite the finding that total serum IgE and IL-5 levels in the "HDM only" trajectory were higher than those in the "pollen predominant" trajectory, the "pollen predominant" trajectory showed a

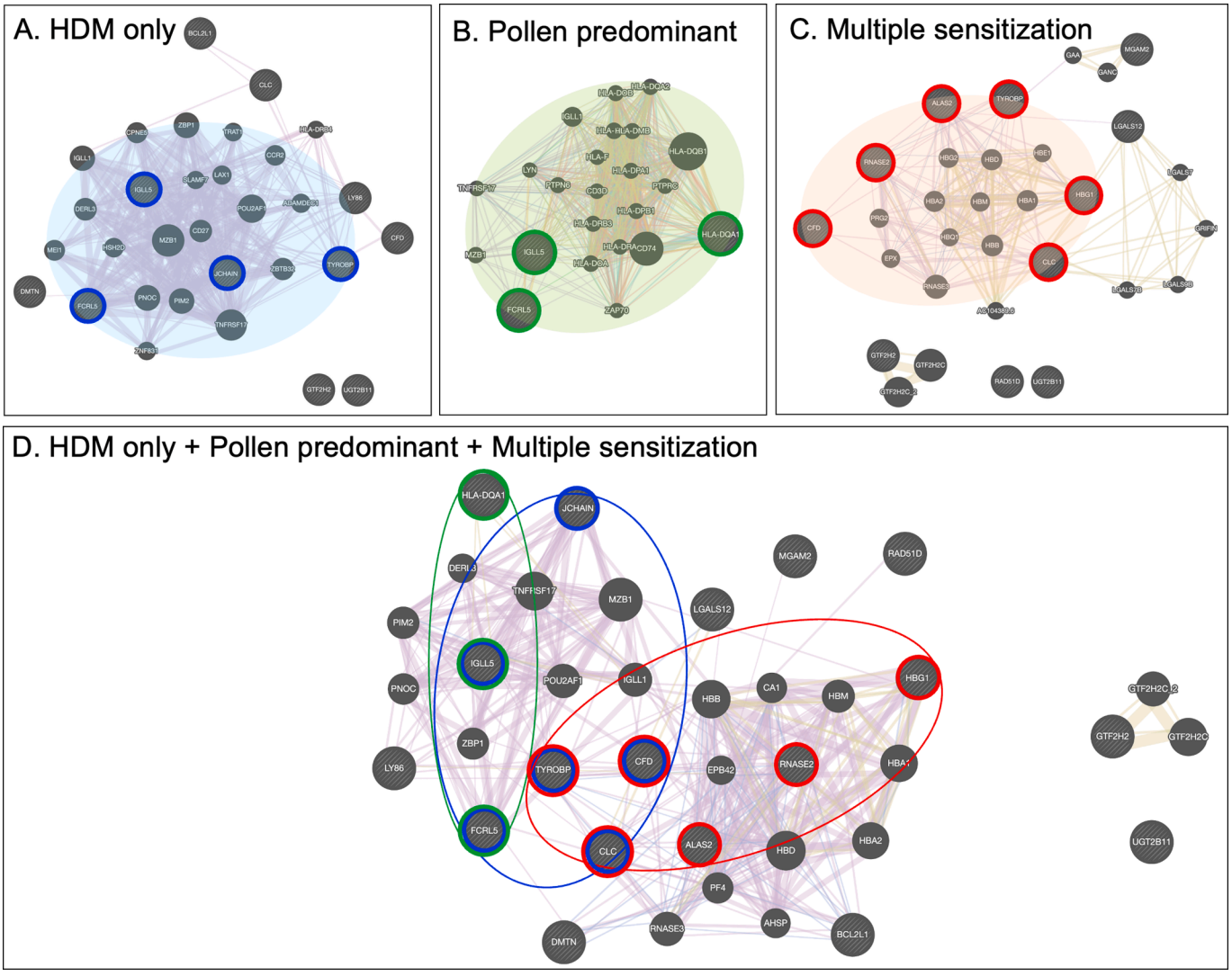

Fig. 4. Public DB-based network analysis based on potential mechanism-cored genes of allergen sensitization trajectories. The GeneMANIA program identified the following hub interactions: **(A)** three cored genes (*IGLL5*, *FCRL5*, and *JCHAIN*) and *TYROBP* in the "HDM only" trajectory; **(B)** one cored gene (*HLA-DQA1*) and two overlapping genes (*IGLL5* and *FCRL5*) in the "HDM only" and "pollen predominant" trajectories; and **(C)** six genes (*TYROBP*, *HBG1*, *CLC*, *CFD*, *RNASE2*, and *ALAS2*) in the "multiple sensitization" trajectory. **(D)** When all of these genes are inputted together, two (*IGLL5* and *FCRL5*) and three (*TYROBP*, *CLC*, and *CFD*) in the "HDM only" trajectory had overlapping interactions with the "pollen predominant" and "multiple sensitization" trajectories, respectively.

stronger association with allergic diseases such as AR and AC during childhood. Although not statistically significant, higher IL-2 levels in the "pollen predominant" trajectory than in the "HDM only" trajectory suggest a partial link to T cell activation, potentially contributing to the characteristic features of allergic diseases within this trajectory. T cell responses to pollen antigens are determined by the specific antigenic epitopes and their binding affinity to MHC class II molecules; these factors contribute to the high allergenicity of pollen.[18] Previous studies[19,20] and our own study confirm that pollen sensitization involves intricate T cell and MHC-mediated mechanisms that are pivotal to initiation and progression of allergic diseases. Given the established role of MHC class II–restricted T cell responses in pollen sensitization, demonstrated in both previous studies[21,22] and our transcriptomic findings, and the nature of pollen as an inhalant allergen,[23] as well as the observed association of the "pollen predominant" trajectory with respiratory allergic diseases, it is plausible that pollen sensitization occurs via trans-airway rather than transdermal exposure.

The 'multiple sensitization' trajectory exhibited not only overlapping signaling pathways with the 'HDM only' and 'pollen predominant' trajectories but also unique enrichment in epithelial barrier dysfunction pathways, such as IL-1 and TGF-β1. IL-1 weakens epithelial integrity by promoting inflammation and disrupting tight junction,[24] while TGF-β1 contributes to barrier dysfunction by inducing epithelial–mesenchymal transition and downregulating tight junction proteins[25,26]; these barrier-disruptive effects may in turn facilitate enhanced allergen penetration and immune activation, thereby promoting multiple allergen sensitizations. Together with exaggerated Th2-driven inflammation, this epithelial vulnerability may predispose children in the "multiple sensitization" trajectory to allergic comorbidities, including AD and FA, leading to more severe and persistent disease phenotypes. Furthermore, the significant increases in IL-5 and IL-2 levels in the "multiple sensitization" trajectory might suggest the interplay between IL-2-driven T cell activation and IL-5-mediated eosinophilic inflammation likely contributes to the heightened risk of allergic

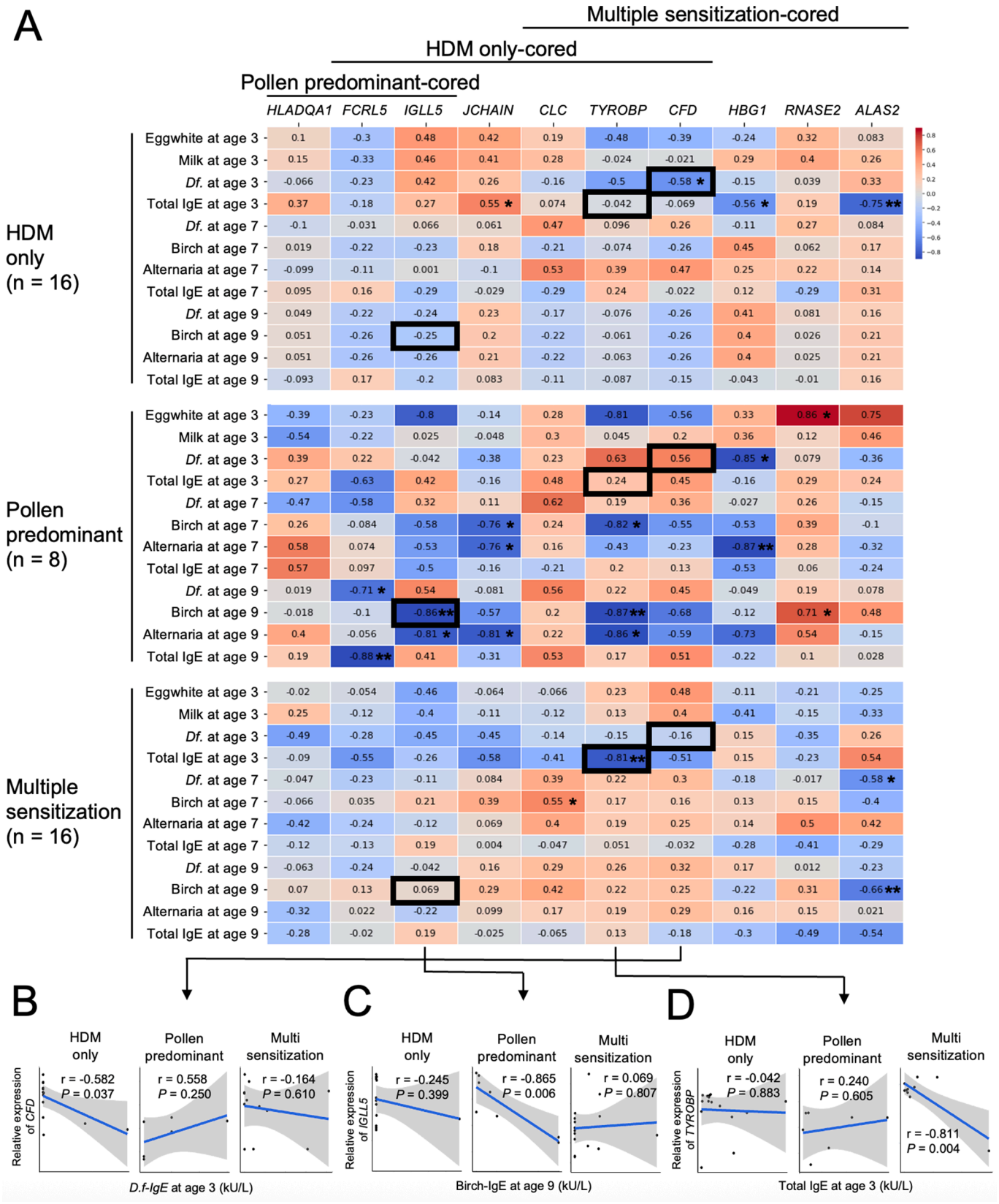

Fig. 5. Correlation between network-cored genes and clinical index. **(A)** Correlations between expression of 10 core genes from each allergen sensitization trajectory and clinical indices at 3, 7, and 9 years of age, $*P < 0.05$ and $**P < 0.01$. Correlations between **(B)** *CFD*, overlapping the "HDM only" and "pollen predominant" trajectories, and *Der p* specific levels at 3 years of age, **(C)** between *IGLL5*, overlapping the "pollen predominant" and "HDM only" trajectories, and birch-specific IgE levels at 9 years of age, and **(D)** between *CFDTYROBP*, overlapping the "pollen predominant" and "HDM only" trajectories, and total serum IgE levels at 3 years of age, are shown. Two-tailed Pearson correlation coefficients (r) and *P*-values were calculated.

diseases with comorbidities in children in this sensitization trajectory.

Previous studies provide clues regarding the role of pathway-involved and network-cored genes in sensitization to specific allergens: (1) *HLA-DQA1* in the "pollen predominant" trajectory is associated with pollen allergens, including mugwort[19,21,27]; (2) *FCRL5* (which along with *IGLL5* was identified in the "HDM only" and "pollen predominant" trajectories) increases after exposure to *Plasmodium falciparum* and grass pollen immunotherapy, indicating its role in B cell and immunoregulatory responses[28–31]; (3) *IGLL5* is involved in memory immune response and inflammation[32]; (4) *CLC* (which along with *TYROBP* and *CFD* was identified in both the "HDM only" and "multiple sensitization" trajectories) produces Charcot-Leyden crystals and is related to various allergens, including HDM and ovalbumin, possibly through mechanisms involving barrier junctions[33–36]; (5) *TYROBP*, also known as *DAP12*, is associated with lipopolysaccharide or bacterial infection, and is a hub gene for immune responses in AR after exposure to allergens, potentially in association of dectin-2[37–39]; and (6) *CFD*, also known as adipsin, was identified in the overlap between "HDM only" and "multiple sensitization" trajectories and appears to be associated with various allergens, particularly food allergens.[40–42]

The significantly enriched C-type lectin receptor, identified in the comparison between "HDM only" and "pollen predominant" trajectories, is known to recognize several components derived from HDM and fungus allergens during the sensitization stage, and plays critical roles in innate immunity and the regulation of adaptive responses.[43] In particular, our DEG analysis showed significantly upregulated expressions of *CLEC4D* and *CLEC4E* (which are involved in C-type lectin receptor signaling) in "HDM only" trajectory compared to "pollen predominant" trajectory (Supplementary Table 8). Meanwhile, we found that IL-1 and tight junctions were enriched in the "multiple sensitization" trajectory compared with the combined "HDM only" and "pollen predominant" trajectories; both IL-1 (α and β) and tight junctions play important roles in promoting allergic sensitization.[44–46] In addition, there is a correlation between IL-1 and tight junction integrity during allergic responses, including allergen sensitization.[47,48] Furthermore, we found that *IGHD*, involved in IL-1 signaling, was downregulated significantly, and *OCLN*, involved in tight junction signaling, was upregulated in the "multiple sensitization" trajectory when compared with the "HDM only" and "pollen predominant" trajectories (Supplementary Table 8). Finally, tight junction signaling, enriched in the "multiple sensitization" trajectory, is related to TGF-β1 signaling, which was also enriched in the "multiple sensitization" trajectory (Supplementary Table 7), suggesting that these associated signaling pathways (IL-1, tight junction, and TGF-β1) may regulate epithelial barrier function in this trajectory.[48–51] Thus, epithelial barrier dysfunction plays a crucial role in the pathogenesis of allergic multi-morbidity during childhood by facilitating sensitization.[52] Further experimental studies are needed to elucidate the causal relationship between epithelial barrier dysfunction and the development of the multiple sensitization.

This study has several limitations. First, we did not assess specific IgE to allergen components; indeed, results derived from whole allergen extracts might partially reflect cross-reactions rather than independent sensitization to specific allergens.[53] Also, we did not include food allergens in the classification of allergen sensitization trajectories because rates of sensitization to food allergens are relatively low, especially in the general population,[54] and there is a limit to the number of allergen groups that can be included in the group-based multi-trajectory model. Instead, we compared levels of specific IgE for egg white and milk across all four identified trajectories at ages 1, 3, and 7 years (Table 3). In addition, although a multiple testing correction using a false discovery rate (FDR) analysis in the transcriptome analysis was performed, the correction failed to reach FDR significance, due to the small sample size of subjects. In addition, due to the cross-sectional nature of the transcriptome analysis, it may remain unclear whether changes in molecular groups are a cause or a consequence of sensitization. However, considering the application of a relatively stringent cut-off for the DEGs, our findings might provide crucial supporting information in the childhood allergic diseases and sensitization, with further needs of functional and replication studies elsewhere in the future.

Even with these limitations, a strength of the study is delineation of allergen sensitization trajectories during childhood and their association with childhood allergic diseases, and identification of the different mechanisms involved. Thus, the data provide a unique opportunity to explore the longitudinal relationships between allergen sensitization trajectories during childhood, allergic outcomes, and their potential mechanisms. As this study was a prospective general population-based birth cohort study, the findings can be generalized to children; however, the data may differ from those reported in studies involving children with severe allergen sensitization.

In conclusion, diverse allergen sensitization trajectories during childhood have characteristic associations with childhood allergic outcomes, and these trajectories have their own characteristic mechanisms although some may be shared. The "multiple sensitization" trajectory showed the strongest associations with all spectra of childhood allergic diseases. Epithelial barrier dysfunction pathways may drive "multiple sensitization", leading to development of diverse allergic diseases during childhood. The results of the present study will help to establish preventive and therapeutic strategies for allergen sensitization and allergic diseases.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.alit.2025.09.007.

Conflict of interest

The authors have no conflict of interest to declare.

Author's contributions

SJH had full access to all of the data used in the study, and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Concept and design: SJH, EL, and JHK.

Data acquisition, analysis, or interpretation: EL, JHK, SYL, HYO, SHL, JoK, EJC, JY, KA, KWK, YHS, DIS, and SJH.

Drafting of the manuscript: SJH, EL, and JHK.

Critical revision of the manuscript for important intellectual content: EL, JHK, SYL, HYO, SHL, JoK, EJC, JY, KA, JiK, KWK, YHS, DIS, JSP, and SJH.

Obtained funding: SJH.

Administrative, technical, or material support: EL, JHK, SYL, HYO, SHL, JoK, EJC, JY, KA, KWK, YHS, DIS, and SJH.

Supervision: SJH, EL, and JHK.

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