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SUPPORTING INFORMATION

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

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De novo sensitization to *Aspergillus fumigatus* in adult asthma over a 10-year observation period

To the Editor,

Although exposure and sensitization to respiratory allergens are key in asthma presentation,¹ few studies have investigated longitudinal changes in sensitization profiles to respiratory allergens among individuals with asthma.² Recently, exposure and sensitization to fungal allergens as risk factors for more persistent, severe asthma have been investigated.³⁻⁶ Considering the chronicity of asthma pathogenesis, changes in sensitization profiles to respiratory allergens can occur temporally and influence prognosis. Physicians sometimes encounter de novo sensitization (DNS) to *Aspergillus fumigatus* (Af), accompanied by allergic bronchopulmonary aspergillosis (ABPA) onset among patients receiving long-term asthma treatment.⁷ Despite its long-term impact on asthma management, risk factors for DNS to Af in asthma patients are unclear. We investigated longitudinal changes in sensitization profiles to respiratory allergens and factors associated with DNS to Af in asthma patients.

Asthma patients treated for ≥ 10 years at the Sagami-hara National Hospital, a large tertiary-care hospital for allergic diseases in central Japan, were assessed. Levels of IgE antibodies (Abs) to allergen extracts from five common fungal allergens and to panels of respiratory allergen components were measured in sera obtained ≥ 10 years ago (1991-2004, **baseline**) and compared with those in recently sampled sera (2015, **follow-up**). Clinical features were compared between asthma patients with or without DNS to Af. Detailed study design (Figure S1) and methods are shown in the supporting information. The ethics committee of Sagami-hara National Hospital approved the study protocol (No. 2016-023), and written informed consent was obtained.

To assess longitudinal changes in sensitization to respiratory allergens, data and sera from 139 subjects were analyzed (Study 1). IgE levels and rates of IgE positivity to respiratory allergens were compared between baseline and follow-up. Serum IgE and IgG levels to Af extract and serum IgE levels to allergen extracts of *Candida albicans*, *Trichophyton rubrum*, *Malassezia* spp., and *Alternaria alternata* were determined using a commercial ImmunoCAP system (Thermo Fisher Scientific, Uppsala, Sweden); IgE levels ≥ 0.35 kU_A/L were considered positive. Serum

levels of IgE specific for native Der f 1 from house dust mites; nCry j 1, Japanese cedar; recombinant Phl p 1 and p 5, timothy-grass; rBet v 1, birch; nAmb a 1, ragweed; nArt v 1, mugwort; rCan f 1, dogs; rFel d 1, cats; rAlt a 1, *Alternaria alternata*; rAsp f 1, f 3, and f 6, Af; rCla h 8, *Cladosporium herbarum*; and rBla g 1, cockroaches were determined using a commercial ImmunoCAP Solid-Phase Allergen Chip (ISAC). IgE levels ≥ 0.3 ISAC standardized units (ISU) were considered positive.

Study 2 involved risk factor analysis for DNS to Af allergen. Nine organic lung disease patients at baseline and/or follow-up were excluded owing to their high susceptibility to *Aspergillus* infection (and accompanying IgE sensitization to Af). After excluding 12 additional patients positive for IgE to Af extract and/or rAsp f 1 at baseline, clinical characteristics of 118 patients were compared between those with or without DNS to rAsp f 1.

Table S1 presents the clinical characteristics of the patients at baseline and follow-up (Study 1). The median interval of serum sampling between baseline and follow-up was 19.1 (IQR, 14.9-21.4) years. Figure 1 and Tables S2 and S3 show the frequencies of positivity for and levels of IgE to each respiratory allergen. Frequencies of patients positive for IgE to Af extract and to rAsp f 1 noticeably increased (from 8.6% to 31% and from 0.7% to 13%, respectively) from baseline to follow-up. Table 1 shows the association between clinical parameters and DNS to rAsp f 1 after adjusting with serum sampling interval (Study 2). Among 118 patients, 14 displayed DNS to rAsp f 1. Male sex, decreased pulmonary function (FEV₁/FVC ratio <65%), nonatopic status at baseline, elevated total IgE levels (>417 IU/mL), and medium-to-high dose of ICS (≥ 500 μ g/day) at follow-up were associated with DNS to rAsp f 1. Among these 14 patients with DNS to rAsp f 1, two and six patients satisfied the diagnostic criteria for ABPA and SAFS, respectively (data not shown).

To our knowledge, this is the first study to clearly document longitudinal changes in respiratory allergen sensitization and identify factors associated with DNS to Af. Notably, IgE positivity frequencies against Af extract and rAsp f 1 increased considerably. Our findings from component-based IgE measurements indicate genuine changes in sensitization profiles to panels of respiratory allergens.⁸

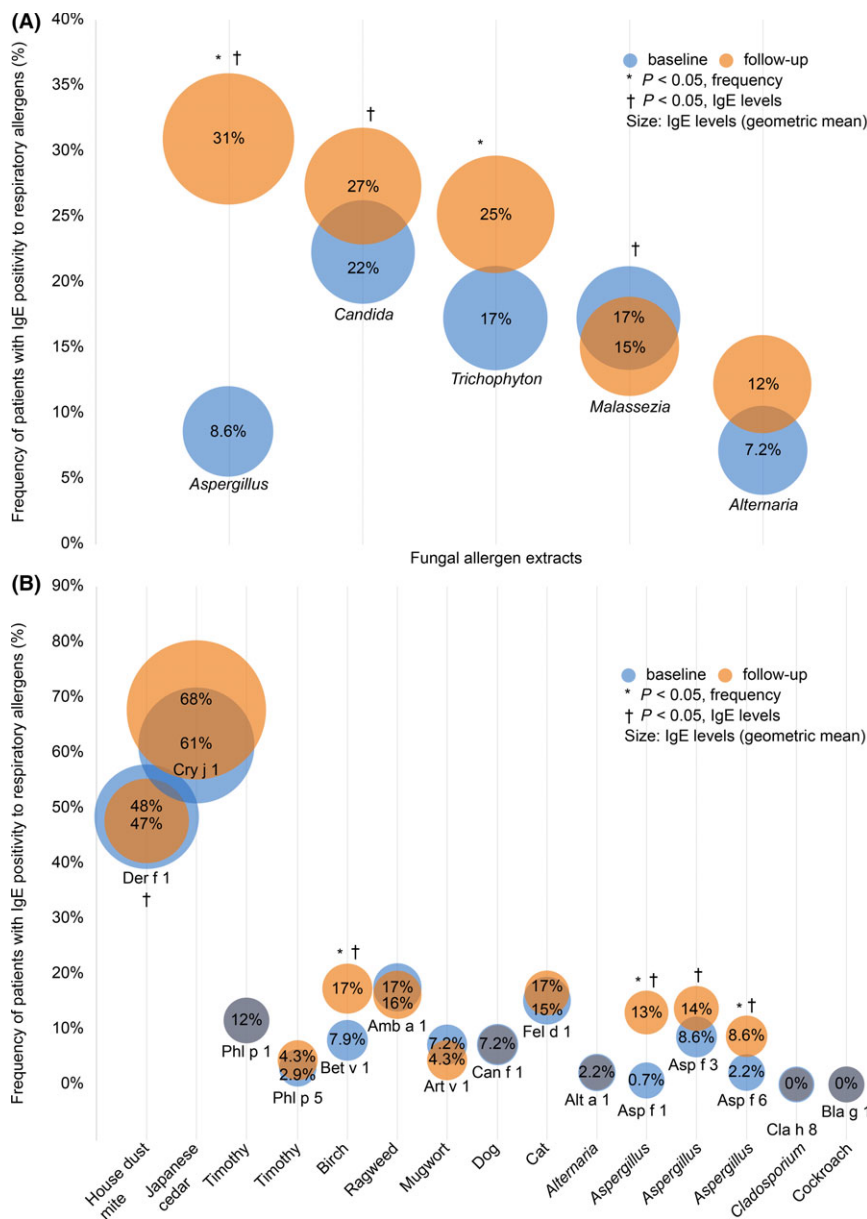


FIGURE 1 The levels and frequency of positivity in IgE Abs to fungal allergen extracts assessed using ImmunoCAP system (A) and respiratory allergen components assessed using ImmunoCAP ISAC (B) at baseline and follow-up. Frequency (circles, y-axis) and IgE levels (diameter) to each respiratory allergen at baseline (blue) and follow-up (red) are indicated. In some species (timothy [Phl p 1], dog, *Alternaria*, *Cladosporium*, and cockroach) in B, the baseline and follow-up bubbles overlap, thus appearing as one. Significant differences are indicated by * $P < 0.05$ for frequency (McNemar's test) and † $P < 0.05$ for IgE levels (Wilcoxon's signed-rank test)

Importantly, Asp f 1 sensitization indicates that these patients have been genuinely exposed and sensitized to Af, which has supposedly germinated in their respiratory tract.⁹

Speculatively, development of fixed airflow obstruction in asthma is consequent upon the damaging effects of airway colonization with Af.³ However, in our study, only decreased pulmonary function at baseline, not pulmonary function at follow-up, was associated with DNS to rAsp f 1. This finding suggests that decreased pulmonary function is a risk factor for Af sensitization and does not result from Af sensitization. The use of medium-to-high dose ICS at follow-up was also associated with DNS to rAsp f 1. Hence, considering the immunosuppressive potential of this medication, high doses of ICS may render susceptibility to Af colonization.¹⁰

Study limitations include the lack of generalizability of our findings to the general asthma population and other allergic respiratory

diseases such as rhinitis. Furthermore, we measured IgE levels at only two time-points (baseline and follow-up) and did not perform sputum or bronchoalveolar lavage assessments of the presence of Af at baseline. Therefore, we can neither conclude with certainty that Af had not colonized at baseline nor pinpoint when colonization and sensitization occurred during long-term observation of ≥ 10 years; this hampers determining the causal relationships between DNS to Af and clinical factors including the use of ICS/OCS. Additionally, clinical data were collected retrospectively, which may have resulted in some bias. Finally, although we excluded patients with organic lung diseases, not all patients underwent CT examination to exclude the possibility of structural lung alterations.

In conclusion, appreciable percentage of patients displayed DNS to Af allergens during observation periods of ≥ 10 years. Physicians should measure anti-Af IgE levels during long-term follow-up of

TABLE 1 Association between clinical parameters and de novo sensitization to rAsp f 1 after adjustment with serum sampling interval

	Adjusted ^a OR (95% CI)
Sex (female)	0.28 (0.09-0.88)
Age at onset (year)	1.00 (0.97-1.03)
Parameters at baseline	
Age (year)	1.02 (0.98-1.06)
Pulmonary function, FEV ₁ /FVC ratio <65% ^b	3.87 (1.19-12.5)
Total IgE >417 IU/mL	0.50 (0.13-1.90)
ICS, FP equivalent ≥500 µg/day	1.66 (0.51-5.46)
GINA treatment step ≥4	1.20 (0.39-3.76)
Atopic (yes)	0.26 (0.08-0.82)
Maintenance OCS treatment (yes)	0.82 (0.17-4.03)
Steroid burst treatment ≥1 time/year	1.38 (0.44-4.27)
Emergency department visits and hospitalization ≥1 time/year	1.17 (0.38-3.62)
Asthma control (GINA)	
Controlled (n = 41)	1
Partly controlled (n = 53)	0.72 (0.17-3.10)
Uncontrolled (n = 24)	2.77 (0.67-11.5)
Parameters at follow-up	
Pulmonary function, FEV ₁ /FVC ratio <65% ^b	0.95 (0.29-3.12)
Total IgE >417 IU/mL	4.74 (1.44-15.5)
ICS, FP equivalent ≥500 µg/day	7.75 (1.62-37.2)
GINA treatment step ≥4	3.67 (0.78-17.4)
Atopic (yes)	0.39 (0.12-1.25)
Maintenance OCS treatment (yes)	1.41 (0.27-7.35)
Treatment with omalizumab (yes)	1.84 (0.19-17.7)
Steroid burst treatment ≥1 time/year	0.98 (0.28-3.40)
Emergency department visits and hospitalization ≥1 time/year	0.88 (0.26-3.07)
Asthma control (GINA)	
Controlled (n = 57)	1
Partly controlled (n = 47)	1.40 (0.43-4.54)
Uncontrolled (n = 14)	0.60 (0.07-5.49)

^aAdjusted with serum sampling interval, not mutually adjusted.

^bThe threshold for FEV₁/FVC ratio was determined on the basis of receiver operator characteristic analysis between FEV₁/FVC ratio and DNS to rAsp f 1.

Results highlighted in bold indicate statistically significant findings. rAsp f 1, recombinant Asp f 1; OR, odds ratio; CI, confidence interval; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; ICS, inhaled corticosteroid; FP, fluticasone propionate; GINA, Global Initiative for Asthma; OCS, oral corticosteroid.

patients with severe asthma, even if previous results were negative, to identify patients with DNS to Af as early as possible.

CONFLICT OF INTEREST

None of the authors have conflict of interests to declare.

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SUPPORTING INFORMATION

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Protective effects of breastfeeding on respiratory symptoms in infants with 17q21 asthma risk variants

To the Editor,

Genetic polymorphisms at the 17q21 locus have been associated with the subsequent onset of childhood asthma and appear to strengthen the association between childhood asthma and early episodes of wheezing.^{1,2} A recent study of Loss et al² showed that 17q21 alleles modified the effect of exposure to older siblings and animal shed on episodes of wheeze in infancy. As environmental factors seem to play a role with respect to the effect modification by the 17q21 polymorphism, our aim was to assess whether the association between asthma-associated 17q21 variants, and lower respiratory symptoms during the 1st year of life, may be modified by breastfeeding. In addition, we investigated whether the described interactions with other environmental exposures, such as older siblings² and tobacco exposure,^{3,4} were reproducible.

We tested our hypothesis within the prospective Basel-Bern Infant Lung Development (BILD) birth cohort of healthy unselected infants ($n = 368$) living in urban environments.⁵ Parental written informed consent was obtained, and the study was approved by the Ethics Committees of Basel and Bern. Respiratory symptoms, such as occurrence of cough, wheeze, or difficulty breathing during the night and day—and their severity—were assessed by weekly telephone interviews using a standardized symptom score (each on a scale of 0-4, with 0 indicating no symptoms and ≥ 3 severe symptoms).⁵ As a primary outcome, the respiratory symptom score was calculated as a sum total of daytime and nighttime symptom scores (on a scale of 0-8).⁵ The secondary outcome was episodes of wheeze in the 1st year of life that were defined as a whistling sound in the chest audible to the parents, or doctor-diagnosed wheeze. Wheeze episodes have been recorded since 2004 on a weekly basis (based on a “yes or no” question); therefore, we restricted our sample to those infants with complete information on wheeze ($n = 252$).

Genomewide genotyping was performed using Illumina HumanOmniExpress Bead Chips (Illumina Inc., San Diego, CA, USA). Five major tagging SNPs at the locus 17q21: rs7216389, rs4795405, rs8079416, rs8065126, and rs3902025 were included in the analysis. These variants were selected as representative of the five highest asthma-associated tagging bins based on unpublished 17q21 fine mapping data (1446 children, 763 asthmatics, from the German MAGIC and ISAAC II studies), presented at the 11th Meeting of the European Human Genetics Societies. For the purposes of our study, either the major tagging SNP from the respective bin (rs3902025) or a proxy in high linkage disequilibrium was analyzed.

Generalized additive mixed model with quasi-Poisson and binomial distribution for count and binary outcomes was used to investigate weekly measured respiratory symptom scores and any breastfeeding (“yes or no” for each week under observation). We applied autoregressive AR(1) modeling to account for interchild variation. Each SNP was coded as 0/1/2 for the number of risk alleles and analyzed separately under the additive model. The interaction was tested by adding to the adjusted model the multiplicative interaction term between breastfeeding and SNP.

Next, we attempted a replication of top SNPs within the Protection against Allergy Study in Rural Environments (PASTURE) birth cohort study ($n = 799$) that was conducted in rural areas. Information on respiratory symptoms (defined as the presence of wheeze or cough) and any breastfeeding was collected from weekly and 4-weekly diaries. We used a stringent Bonferroni P -value correction threshold of 0.01 (0.05/5) and 0.025 (0.05/2) for discovery and replication analysis, respectively. Further information on demographic (eTable 1) and genotype characteristics, methods, and meta-analyses of both cohorts is provided in the Supporting Information.

The 17q21 SNPs were not associated with respiratory symptom score during the 1st year of life. When we stratified infants by breastfeeding status, we found that, during those weeks when infants were breastfed, the carriers of asthma risk alleles of the

*Members of the BILD and PASTURE study groups are listed in the Appendix.

Supporting Information

Supplementary methods

Study design

We assessed patients with asthma, who had visited the Allergology Department of Sagami-hara National Hospital, one of the largest tertiary-care hospitals for allergic diseases in central Japan, for ≥ 10 years and whose serum samples, collected 10 years ago or earlier, were available. Numerous serum samples from asthma patients have been obtained with patient consent and stored for decades.

Figure S1 shows the study flow. First, a list of outpatients with asthma at the Sagami-hara National Hospital between January 2015 and June 2015 was prepared ($n = 747$). Among these, we identified 346 patients with available sera sampled between January 1991 and December 2004 (**baseline**). Through medical chart reviews, we excluded patients whose pulmonary function data ($n = 102$) and clinical information including treatment drugs, steroid burst treatment times, and emergency department visit and hospitalization times ($n = 83$) for the baseline blood sampling were unavailable and obtained 161 candidates for this study. If stored sera after January 2015 (**follow-up**) were available, they were used for this study. If conserved sera at follow-up were not available, serum samples were taken during outpatient visits, with written informed consent of the patients ($n = 10$) in accordance with the tenets of the Declaration of Helsinki. Finally, 139 subjects were assessed for Study 1 ($n = 139$). The study protocol (No.2016-023) was approved by the Ethics Committee of Sagami-hara National Hospital.

Study 1: Analysis of longitudinal changes in the sensitization to respiratory allergens

Sera and clinical data between January 1991 and December 2004 were considered **baseline**, and those after January 2015 were considered **follow-up**. Between baseline and follow-up, we compared the IgE levels and rates of IgE positivity to respiratory allergens. In patients who had more than one conserved serum sample between January 1991 and December 2004, the oldest serum sample was used for the analysis. Consequently, most sera at baseline were collected and conserved as a work-up for the sensitization profile at the initial visit.

Through medical chart reviews, we also obtained information on the dose of inhaled corticosteroids (ICS), use of maintenance oral corticosteroids (OCS), treatment step and asthma control based on the GINA 2015 guidelines (1) during one month after the day of serum sampling, and the number of steroid burst treatments and emergency department visits and hospitalizations during one year after the day when the serum was sampled. After omitting short-acting inhaled bronchodilators for 4 h and long-acting oral and inhaled agents for 12 h prior to attendance, pulmonary function was measured using a spirometer (Auto Spiro AS-303; Minato Medical Science Co., Ltd., Osaka, Japan). Spirometry data obtained within 6 months from the day when the serum was sampled were used for the analysis.

Study 2: Risk factor analysis for de novo sensitization to *Aspergillus fumigatus* allergen

Study 2 aimed to identify risk factors for de novo sensitization (DNS) to *Aspergillus fumigatus* (Af) allergen between the baseline and follow-up. We excluded from the analysis patients with organic lung diseases (bronchiectasis [$n = 1$], post-tuberculosis sequelae [$n = 3$], chronic bronchitis [$n = 1$], non-

tuberculous mycobacterial infection [n = 1], COPD [n = 1], chronic eosinophilic pneumonia [n = 1], and eosinophilic granulomatosis with polyangiitis [n = 1]) at baseline and/or follow-up, because the risk of *Aspergillus* infection (and supposedly the accompanying IgE sensitization to Af) is high in such patients.(2,3) After excluding 12 additional patients who were positive for IgE to Af extract (using ImmunoCAP, ≥ 0.35 kU_A/L) and/or recombinant Asp f 1 (using ImmunoCAP Solid-Phase Allergen Chip [ISAC], ≥ 0.3 ISAC standardized units [ISU]) at baseline, the clinical characteristics of patients with DNS to rAsp f 1 were compared with those of patients who did not present sensitization.

Serological analysis

Serum samples from patients at baseline and follow-up were stored at -20°C until assayed. Serum IgE and IgG levels to Af extract and serum IgE levels to extracts of *Candida albicans*, *Trichophyton rubrum*, *Malassezia spp.*, and *Alternaria alternata* were measured using a commercial ImmunoCAP system (Thermo Fisher Scientific, Uppsala, Sweden). IgE levels of ≥ 0.35 kU_A/L were considered positive. Serum levels of IgE specific for native Der f 1 from house dust mites (*Dermatophagoides farinae*), nCry j 1 from the Japanese cedar (*Cryptomeria japonica*), rPhl p 1 and p 5 from timothy-grass (*Phleum pratense*), rBet v 1 from birch (*Betula verrucosa*), nAmb a 1 from ragweed (*Ambrosia artemisiifolia*), nArt v 1 from mugwort (*Artemisia vulgaris*), rCan f 1 from dogs (*Canis familiaris*), rFel d 1 from cats (*Felis domesticus*), rAlt a 1 from *Alternaria alternata*, rAsp f 1, f 3, and f 6 from Af, rCla h 8 from *Cladosporium herbarum*, and rBla g 1 from the cockroach (*Blattella germanica*) were determined using a commercial ImmunoCAP ISAC system. Atopy was defined as scoring positive for at least one of the aforementioned respiratory allergens. Serum testing for total IgE levels (IU/mL) was performed using the ImmunoCAP total IgE test.

Diagnostic criteria for asthma, severe asthma with fungal sensitization, and allergic bronchopulmonary aspergillosis

Asthma was diagnosed on the basis of the guidelines of the American Thoracic Society (ATS) and was determined as episodes of paroxysmal dyspnea, wheezing, and cough and increased bronchial hyperresponsiveness.(4)

Severe asthma with fungal sensitization (SAFS) was defined as meeting all of the following requirements: (I) high dose of ICS (fluticasone-equivalent daily dose ≥ 500 μg), continuous OCS (at least 5 mg of prednisolone or its equivalent for at least 6 months), or at least 4–6 courses of oral/intravenous corticosteroids over the previous 12 or 24 months, (II) elevated serum IgE levels to any fungal allergens (including allergen components), (III) total IgE levels $< 1,000$ IU/mL, and (IV) absence of serum IgG to Af (≤ 40 mgA/L).(5,6)

An allergic bronchopulmonary aspergillosis (ABPA) was diagnosed in accordance with the most widely accepted diagnostic criteria established by Rosenberg-Patterson et al.(7,8) with minor modifications, namely: (i) asthma, (ii) immediate cutaneous reactivity to Af, (iii) total IgE levels > 417 IU/mL, (iv) elevated serum IgE levels to Af, (v) central bronchiectasis, (vi) chest radiographic infiltrates, (vii) serum IgG-positivity to Af (> 40 mgA/L, as a substitute for precipitating Abs), and (viii) peripheral blood eosinophilia (> 1000 counts/ μL). Serum IgE testing, rather than skin tests, are routinely used for screening for respiratory allergen sensitization in asthma patients. Therefore, all

patients testing positive for serum IgE to Af (≥ 0.35 kU_A/L) were considered to satisfy both criteria (ii) and (iv).⁽⁹⁾ We defined patients with ABPA as those meeting criteria (i)–(iv) and more than six of the eight ABPA criteria.

Statistical analysis

The data were analyzed using SPSS ver. 22.0 (IBM, Tokyo, Japan). IgE levels and rates of IgE positivity for each respiratory allergen were compared between baseline and follow-up. The demographic characteristics, total IgE levels, pulmonary function, asthma control, and treatment step, assessed per GINA 2015 guidelines,⁽¹⁾ were also compared between baseline and follow-up. A paired *t*-test, Wilcoxon signed-rank test, or McNemar's test was used to analyze the statistical significance of differences between baseline and follow-up values. The association between clinical parameters and DNS to rAsp f 1 was evaluated using multivariate logistic regression analysis, after adjusting with serum sampling interval. P-values less than 0.05 were considered statistically significant.

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Supplementary tables

TABLE S1. Demographic characteristics at baseline and follow-up

	Baseline (n = 139)	Follow-up (n = 139)	P-value
Sex (female), n (%)		96 (69.1)	—
Age at onset, mean ± SD		27.7 ± 18.3	—
Age, mean ± SD	43.7 ± 14.2	61.5 ± 14.3	—
Disease duration (year), mean ± SD	16.1 ± 13.2	33.8 ± 14.6	—
Smoking history (yes), n (%)	40 (28.8)	40 (28.8)	—
Pack year for smokers, median (IQR)	10.7 (5.00–26.8)	11.6 (5.13–30.0)	0.008
Total IgE (IU/mL), median (IQR)	263 (78.0–762)	261 (78.2–654)	0.354
IgG to Af (mgA/L), median (IQR)	22.0 (10.2–40.3)	31.8 (17.4–53.3)	<0.001
Atopic, n (%)	100 (71.9)	105 (75.5)	0.302
Body mass index (kg/m ²), Mean ± SD	22.4 ± 3.29	22.9 ± 3.80	0.005
Pulmonary function			
FEV ₁ /FVC ratio, mean ± SD	69.7 ± 12.0	68.6 ± 11.5	0.178
FEV ₁ % predicted, mean ± SD	78.6 ± 20.0	85.6 ± 21.6	0.006
ICS (FP equivalent) ≥500 µg/day, n (%)	36 (25.9)	73 (52.5)	0.001
GINA treatment step ≥4, n (%)	53 (38.1)	90 (64.7)	<0.001
Maintenance OCS treatment (yes), n (%)	21 (15.1)	15 (10.8)	0.316
Treatment with omalizumab, n (%)	0 (0)	6 (4.3)	—
Steroid burst treatment, times per year ≥1, n (%)	60 (43.2)	36 (27.3)	<0.001
Emergency department visits and hospitalizations per year ≥1, n (%)	63 (45.3)	38 (27.3)	<0.001
Asthma control (GINA), n (controlled: partly controlled: uncontrolled)	48 : 62 : 29	69 : 55 : 15	0.001

SD, standard deviation; IQR, interquartile range; Af, *Aspergillus fumigatus*; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; ICS, inhaled corticosteroid; FP, fluticasone propionate; GINA, Global Initiative for Asthma; OCS, oral corticosteroid

TABLE S2. The levels and frequency of positivity in IgE Abs to fungal allergen extracts assessed using ImmunoCAP system

Fungal allergen extracts	IgE levels, geometric mean (size of bubble)		Frequency of positivity (Vertical axis)	
	Baseline (kU _A /L)	Follow-up (kU _A /L)	Baseline (%)	Follow-up (%)
<i>Aspergillus</i>	0.137	0.292 [†]	8.6	31*
<i>Candida</i>	0.179	0.227 [†]	22	27
<i>Trichophyton</i>	0.182	0.229	17	25*
<i>Malassezia</i>	0.179	0.166 [†]	17	15
<i>Alternaria</i>	0.134	0.157	7.2	12

[†]p <0.05, IgE levels between baseline and follow-up using Wilcoxon signed-rank test

*p <0.05, frequency between baseline and follow-up using McNemar's test

TABLE S3. The levels and frequency of positivity in IgE Abs to respiratory allergen components assessed using ImmunoCAP ISAC

Allergen components	IgE levels, geometric mean (size of bubble)		Frequency of positivity (Vertical axis)	
	Baseline (ISU)	Follow-up (ISU)	Baseline (%)	Follow-up (%)
Der f 1	0.840	0.557 [†]	48	47
Cry j 1	1.069	1.516	61	68
Phl p 1	0.162	0.166	12	12
Phl p 5	0.114	0.124	2.9	4.3
Bet v 1	0.141	0.221 [†]	7.9	17*
Amb a 1	0.183	0.176	17	16
Art v 1	0.126	0.121	7.2	4.3
Can f 1	0.132	0.125	7.2	7.2
Fel d 1	0.174	0.158	15	17
Alt a 1	0.108	0.107	2.2	2.2
Asp f 1	0.101	0.154 [†]	0.7	13*
Asp f 3	0.126	0.162 [†]	8.6	14
Asp f 6	0.104	0.131 [†]	2.2	8.6*
Cla h 8	0.100	0.100	0	0
Bla g 1	0.100	0.100	0	0

ISU, ISAC standardized units

[†]p <0.05, IgE levels between baseline and follow-up using Wilcoxon signed-rank test

*p <0.05, frequency between baseline and follow-up using McNemar's test

Supplementary figure

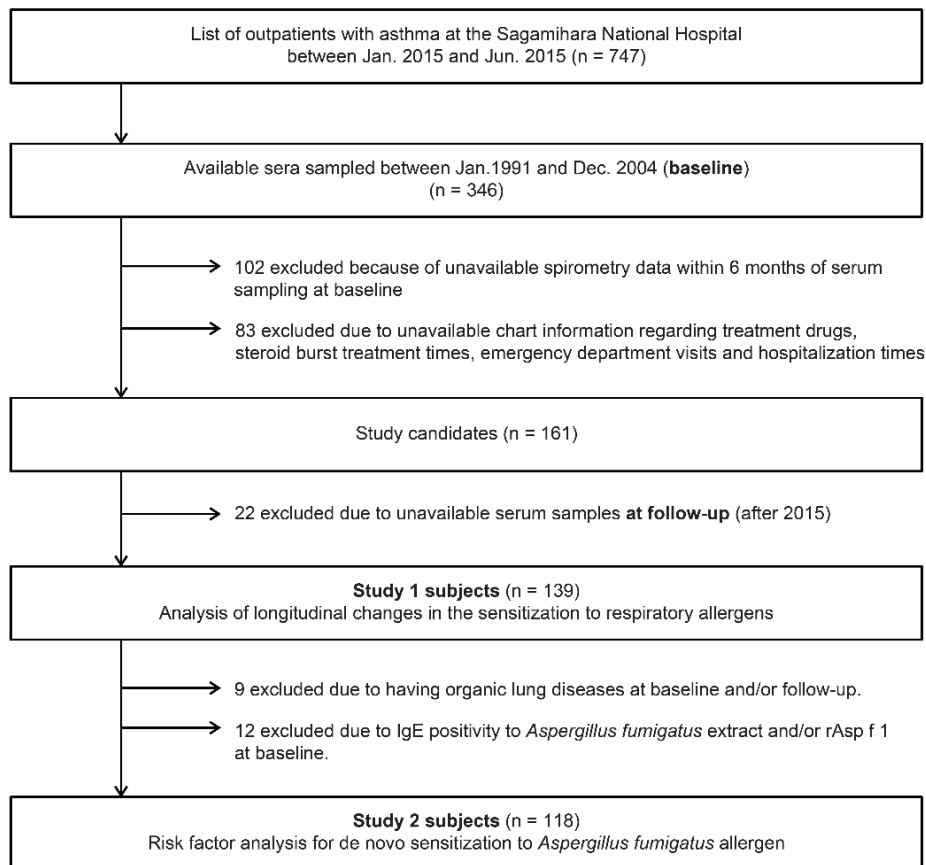


Figure S1. A flow diagram of the study design