

Allergen extract vs. component sensitization and airway inflammation, responsiveness and new-onset respiratory disease

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Summary

Background The absence of IgE sensitization to allergen components in the presence of sensitization to the corresponding extract has been reported, but its clinical importance has not been studied.

Objective To evaluate the clinical significance of IgE sensitization to three aeroallergen extracts and the corresponding components in relation to the development of respiratory disease.

Methods A total of 467 adults participated in the European Community Respiratory Health Survey (ECRHS) II and 302 in ECRHS III, 12 years later. IgE sensitization to allergen extract and components, exhaled nitric oxide (FeNO) and bronchial responsiveness to methacholine were measured in ECRHS II. Rhinitis and asthma symptoms were questionnaire-assessed in both ECRHS II and III.

Results A good overall correlation was found between IgE sensitization to extract and components for cat ($r = 0.83$), timothy ($r = 0.96$) and birch ($r = 0.95$). However, a substantial proportion of subjects tested IgE positive for cat and timothy allergen extracts but negative for the corresponding components (48% and 21%, respectively). Subjects sensitized to both cat extract and components had higher FeNO ($P = 0.008$) and more bronchial responsiveness ($P = 0.002$) than subjects sensitized only to the extract. Further, subjects sensitized to cat components were more likely to develop asthma ($P = 0.005$) and rhinitis ($P = 0.007$) than subjects sensitized only to cat extract.

Conclusion Measurement of IgE sensitization to cat allergen components would seem to have a higher clinical value than extract-based measurement, as it related better to airway inflammation and responsiveness and had a higher prognostic value for the development of asthma and rhinitis over a 12-year period.

Keywords allergen components, asthma, extract based, FeNO, rhinitis

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Introduction

The prevalence of allergic diseases is still on the rise in many parts of the world, and it is estimated that 25% of the population worldwide suffers from allergies [1]. Current tools for the detection of IgE-mediated sensitization are based on allergen extracts and used for both *in vivo* skin testing and *in vitro* IgE testing [2]. During the last decade, component-resolved IgE antibody determination, either as single measurement [3] or by

means of microarray techniques [3] that allow IgE testing of more than one hundred allergen components, has been introduced into the field of clinical Allergology [3, 4]. The clinical usefulness of these new methods is under validation, but they are considered to allow for more precise allergy diagnosis and to distinguish between genuine IgE sensitizations to a certain allergen source and sensitizations attributable to cross-reactivity [4–6]. This in turn facilitates the choice of patients appropriate for immunotherapy and giving advice on

targeted exposure reduction or on the need to perform food challenges [7]. However, the importance of IgE sensitization to allergen components in relation to respiratory disease and especially new-onset of respiratory disease has been scarcely studied.

Several child cohort studies [8–10] have examined sensitization using both extract-based tests and allergen components, although none of these studies included a direct comparison. Only a few epidemiological studies [11–13] have compared IgE testing towards allergen extracts with testing towards allergen components, and all these studies have had a cross-sectional design [14]. There is generally a high concordance between single and multiplex measurements of IgE to allergen components [11], as well as between measurements using allergen extract and the corresponding components [15–20]. However, differences have been reported for some allergens [12, 13], with a subgroup of subjects being sensitized according to extract-based measurements, but not according to measurements using components. The clinical importance of this discordance has not been studied in relation to present airway inflammation and responsiveness or in relation to later development of allergic airway disease.

IgE sensitization to aeroallergen extracts has been linked to increased airway inflammation, systemic eosinophilic inflammation and bronchial responsiveness [21, 22]. We have previously reported that sensitization to components of seasonal allergens relates to asthma symptoms and sensitization to components of perennial allergens relates to asthma symptoms, airway inflammation and bronchial responsiveness [23]. Prosperi et al. [14] reported that IgE to dust mite, pollen and pet components were highly associated with asthma, and that IgE to pollen and dust mite were associated with rhino-conjunctivitis. Few studies have examined the prognostic value of IgE sensitization for later onset of respiratory disease [24], and only one study in children examined IgE sensitization to timothy allergen components in relation to new-onset rhinitis [25].

The aim of this study was to compare the performance of extract-based and component-based measurements of IgE sensitization to cat, timothy and birch, in relation to current and new-onset rhinitis and asthma, as well as in relation to airway inflammation and responsiveness.

Methods

Study population

The European Community Respiratory Health Survey (ECRHS) has been presented in detail elsewhere [26]. This study was based on 451 subjects from Uppsala

centre, who participated in the ECRHS II, which was a 9-year follow-up of the first ECRHS [27], and 302 subjects from the ECRHS III, which was the follow-up of ECRHS II (Fig. S1). A percentage of 35% of the baseline population was lost at follow-up, but they had similar characteristics as the subjects who were followed up (Table S1A).

This study focused on 302 people who were examined with both allergen extract (ImmunoCAP IgE) and allergen components (ImmunoCAP ISAC) at baseline and followed up 12 years later with a questionnaire about respiratory symptoms and disease in the ECRHS III.

Questionnaires

The ECRHS II & III main questionnaires (<http://www.ecrhs.org>) [27] were used to obtain information about respiratory symptoms, exposure to investigated allergens and smoking history.

Diagnosis of rhinitis and asthma

A person was considered to have allergic rhinitis if he/she gave a positive answer to the question: 'Do you have any nasal allergies, including hay fever?' [28].

A person was recorded as having asthma if he/she had ever been diagnosed with asthma and had an asthma attack or one of the following symptoms during the last 12 months before assessment: wheeze, nocturnal chest tightness or attack of shortness of breath [29].

IgE sensitization

The presence of IgE antibodies was examined using both an allergen extract-based method [30] for birch, timothy, *Dermatophagoides pteronyssinus*, *Cladosporium herbarum* and cat (ImmunoCAP system: Immunodiagnosics, Thermo Fisher Scientific, Uppsala, Sweden) and chip technology (ImmunoCAP ISAC 103; Thermo Fischer Scientific) [31]. *Dermatophagoides pteronyssinus* and *Cladosporium herbarum* are not presented in the present paper due to the small number of sensitized subjects to the respective component allergens (3.2% and 0.2%, as previously reported [23]). The subanalyses focusing on the groups with negative sensitization to allergen components but positive to extract were carried out only for timothy and cat, as this group was very small for birch ($n = 11$). A subject was defined as IgE-sensitized to an allergen extract in case of IgE values ≥ 0.35 kU/L for this allergen [32]. Subjects with IgE antibody levels < 0.35 kU/L (the lower limit of detection in ECRHS II) were given an arbitrary value of 0.17 kU/L. Regarding component sensitization, subjects were considered non-IgE-sensitized if the

Table 1. Characteristics and type of sensitization in subjects divided into groups based on their respiratory disease status at baseline (cut-off limit for allergen components 0.30 ISU and for allergen extract 0.35 kU/L)

	Healthy	Only rhinitis	<i>P</i> value*	Asthma ± Rhinitis	<i>P</i> value*	<i>P</i> value†
<i>N</i>	208	147		96		
Age (years)	43.6 ± 7.3	42.5 ± 7.2	0.54	40.5 ± 7.3	0.002	0.09
Female sex (%)	41.8	49.7	0.68	52.1	0.095	0.71
BMI (kg/m ²)	25.2 ± 3.9	24.7 ± 3.9	0.77	25.5 ± 4.6	1	0.40
FeNO (ppb)	18.3 (16.7, 19.9)	20.4 (17.7, 23.5)	0.5	25.1 (20.3, 30.9)	0.006	0.18
S-ECP (mg/L)	7.2 (6.6, 7.9)	7.8 (7.0, 8.8)	0.82	9.7 (8.5, 11.0)	0.001	0.041
Slope (units)	7.6 (7.3, 7.9)	7.4 (6.9, 7.8)	1	5.4 (4.9, 6.0)	<0.001	<0.001
Allergen components						
<i>Birch (any)</i> (%)	8.7	27.2	<0.001	49.0	<0.001	0.001
rBet v 1 (%)	8.2	27.2	<0.001	47.9	<0.001	0.001
rBet v 2 (%)	1.0	0.7	0.78	4.2	0.19	0.14
rBet v 4 (%)	0.5	0	0.4	4.2	0.019	0.013
<i>Timothy (any)</i> (%)	8.2	19.7	0.001	47.9	<0.001	<0.001
rPhl p 1 (%)	3.4	13.6	<0.001	40.6	<0.001	<0.001
rPhl p 2 (%)	1.9	6.8	0.02	18.8	<0.001	0.004
nPhl p 4 (%)	2.9	7.5	0.046	24.0	<0.001	<0.001
rPhl p 5 (%)	1.4	12.9	<0.001	26.0	<0.001	0.009
rPhl p 6 (%)	1.4	7.5	0.028	20.8	<0.001	0.002
rPhl p 7 (%)	0.5	0	0.4	2.1	0.19	0.08
rPhl p 11 (%)	1.0	6.1	0.006	6.3	0.007	0.97
rPhl p 12 (%)	1.0	0	0.23	2.1	0.43	0.08
<i>Cat (any)</i> (%)	5.3	11.6	0.031	36.5	<0.001	<0.001
rFel d 1 (%)	3.9	6.8	0.21	27.1	<0.001	<0.001
nFel d 2 (%)	1.4	3.4	0.22	6.3	0.022	0.30
rFel d 4 (%)	1.9	5.4	0.07	21.9	<0.001	<0.001
Allergen extract						
Birch (%)	7.2	18.4	0.003	48.3	<0.001	<0.001
Timothy (%)	8.7	27.9	<0.001	50.0	<0.001	<0.001
Cat (%)	11.1	24.5	0.001	58.3	<0.001	<0.001

*Compared with subjects without asthma and rhinitis.

†Compared with subjects with only rhinitis.

signal was non-measurable or very low (<0.3 ISU). In order to get an assessment of the degree of IgE sensitization to components of timothy, birch and cat, the IgE antibody levels against the components of each allergenic source were summed up (Sum IgE) for each of these three allergenic sources [30]. Subjects with non-detectable IgE antibody levels to any of the components of an allergen were given an arbitrary value of 0.05 ISU for Sum IgE for each respective allergen. The components analysed for each allergen are given in Tables 1 and S1B (for cut-off limit 0.1 ISU for allergen components). We used data on IgE sensitization against Bet v 2, Mer a 1, Hev b 8 and Phl p 12 to define a profilin reactive subset and corresponding data for Bet v 4 and Phl p 7 to define a polcalcin reactive subset of the participants.

Measurements of exhaled NO

The fraction of exhaled nitric oxide (FeNO) was measured in ECRHS II (baseline) at an exhalation flow rate

of 50 mL/sin accordance with ATS/ERS recommendations [33], using a chemiluminescence analyzer (Sievers NOA 280; Sievers, Boulder, CO, USA), as presented previously [23].

Methacholine challenge test

Methacholine was delivered via a Mefar dosimeter (Mefar; Bovezzo, Italy). Details of the methacholine challenge test have been described elsewhere [21]. The level of bronchial responsiveness measured in ECRHS II (baseline) was expressed using a dose-response slope [34], where a lower value indicates a higher degree of bronchial responsiveness.

Eosinophil cationic protein

The blood samples collected in ECRHS II (baseline) for the measurement of serum eosinophil cationic protein (S-ECP) were allowed to coagulate for 60 min at 24°C before they were centrifuged. Sera were stored at

–20°C. The concentration of S-ECP was assayed with a double-antibody radioimmunoassay (Thermo Fisher Scientific, Uppsala, Sweden).

Statistical methods

Statistical analyses were performed using STATA 11.0 software (Stata Corp., 2001, TX, USA). FeNO, S-ECP values and IgE antibody concentrations (both methods of assessment) were log-transformed before statistical analysis. Chi-squared tests and unpaired t-tests were used when comparing (a) healthy vs. rhinitis vs. asthmatics or (b) subjects without sensitization vs. subjects sensitized only to allergen extract vs. subjects sensitized to both techniques. No corrections for multiple comparisons were applied. Linear regression was performed to assess correlation between log-transformed titres of IgE for allergen extract and sum of IgE for different components for the corresponding allergen using multiplex technique. To evaluate the agreement between allergen extract and component IgE testing, calculations were made of the positive per cent agreement (PPA), that is the percentage of subjects positive to allergen components (>0.3 ISU) out of all subjects who were positive using the extract-based method at an either cut-off of >0.35 kUA/L or an arbitrary chosen [12, 13] higher cut-off of >1 kUA/L, and of the negative per cent agreement (NPA), that is the percentage of subjects who were negative to allergen components (<0.3 ISU) out of all subjects who were negative to the extract. PPA and NPA were calculated also for cut-off 0.1 ISU for allergen components and 0.35 kUA/L for the extract-based method.

Simple and multiple linear regressions were used when analysing correlation between the type of sensitization and exhaled NO and correlation between sensitization and bronchial responsiveness. Simple and multiple logistic regressions were used to analyse the effect of the type of sensitization on new-onset asthma and new-onset rhinitis. Differences in Pseudo-R² values in the aforementioned models were also reported. A *P* value of <0.05 was considered statistically significant.

Ethics

All subjects gave their permission for the utilization of personal data for the purpose of this study. The study was approved by the Ethics Committee at the Medical Faculty at Uppsala University (Dnr 1999/313 and 2010/068).

Results

Patient characteristics

The characteristics of the subjects, divided into groups based on rhinitis and asthma status at baseline (ECRHS

II), are given in Table 1. Subjects with asthma were younger and had higher FeNO and S-ECP, and more bronchial responsiveness, than subjects without asthma and rhinitis. Asthmatics had higher S-ECP and bronchial responsiveness than subjects with only rhinitis.

IgE sensitization in relation to asthma and rhinitis

A similar profile was seen for IgE sensitization to birch, timothy and cat, with higher prevalence among rhinitis subjects than healthy subjects and a further increased prevalence in subjects with asthma. Furthermore, this was consistent for IgE sensitization assessed by means of allergen components for a cut-off of both 0.3 ISU (Table 1) and 0.1 ISU (Table S1B) and extract for a cut-off of both 0.35 kU/L (Table 1) and 1 kU/L (Table S2).

Distribution of sensitization to allergen components

For timothy, the three most common components were Phl p 1, Phl p 4 and Phl p 5 and had similar prevalence (Fig. S2a). IgE sensitization to Fel d 1 and Fel d 4 dominated among cat components (Fig. S2b). Birch is not shown as Bet v 1 dominated in the birch-sensitized subjects (98% of subjects sensitized to any birch component).

IgE sensitization assessed by analysis using allergen extracts and components

Generally, more subjects were sensitized to allergen extracts than to allergen components, for all investigated allergens (Fig. 1). In subjects with total IgE <50 kU/L, prevalence of sensitization (cut-off limit 0.3 ISU) against any cat component was 1.9% vs. 28.9% in subjects with total IgE ≥ 50 kU/L (*P* < 0.001) for cat and for timothy components were 7.4% vs. 37.1% (*P* < 0.001), respectively (Table S3A and B). The same pattern was observed for each cat and timothy components (data not shown).

A total of 48% of subjects who were sensitized to cat extract were not sensitized to any investigated cat allergen component (PPA=52%, NPA: 100%). The 56 individuals who were positive for the cat extract but negative for the cat allergen components had mostly low IgE levels to cat, with a geometrical mean of 1.3 (1.0, 1.7) kU/L, vs. 5.6 (4.3, 7.3) kU/L in subjects IgE-sensitized to both cat extract and components (Fig. S3a). After choosing a higher cut-off for being sensitized to allergen extract (1 kU/L), 34% of individuals positive to cat extract (according to the higher cut-off) were still not sensitized to any investigated cat component (PPA = 66%; Table S3C). This high percentage of individuals not sensitized to cat components decreased to 23% for cut-off >2 kU/L IgE

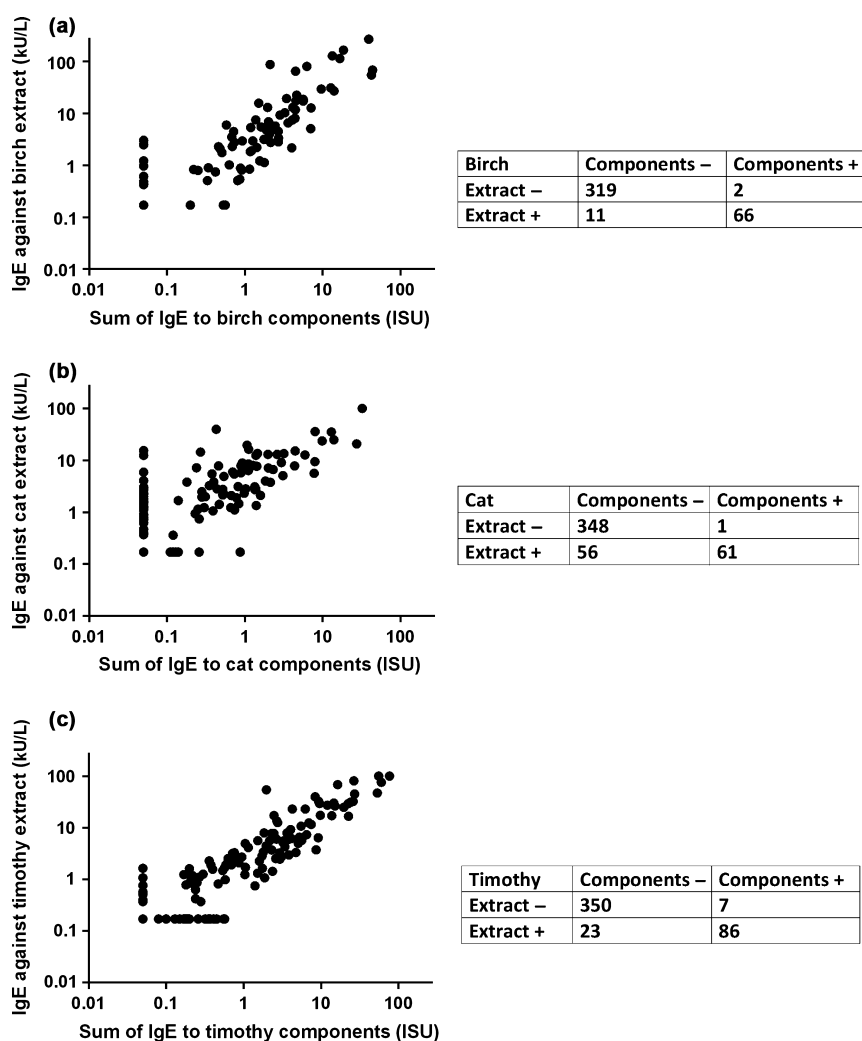


Fig. 1. Contingency tables and scatter plots of IgE extract (Y-axis) vs. sum of components (X-axis) (both axes log-scales). The number of sensitized subjects for each allergen according to each method is presented in the table in each panel (a) birch, (b) cat, (c) timothy.

against cat extract and to 16% for cut-off >3.5 kU/L IgE against cat extract (Table S3D, Fig. S3). However, we still have a discordant group that had same levels of IgE against cat extract, FeNO and ECP, but lower levels of bronchial reactivity compared to the group with subjects positive to both cat extract and any cat component (data not shown). If we used cut-off limit 0.1 ISU for components, then we had 46 individuals who were positive for the cat extract but negative for the cat allergen components, with a geometrical mean of 1.2 (0.9, 1.6) kU/L vs. 4.8 (3.7, 6.3) kU/L in subjects IgE-sensitized to both cat extract and components. For this lower cut-off limit for cat components (>0.1 ISU) and for cut-off limit 0.35 kU/L for extract, the PPA and NPA were 61% and 97%, respectively (Table S3). Using the lower cut-off limit for cat components (>0.1 ISU) gave the same independent associations in the multivariate models of FeNO, bronchial responsiveness, new-onset rhinitis and new-onset asthma as the

cut-off limit (0.3 ISU) for the cat components (data not shown).

For timothy, 21% of the subjects who were sensitized to timothy extract were not sensitized to timothy components (PPA = 79%, NPA = 98%). The highest agreement was seen for birch, for which only 14% of the extract-sensitized subjects were not sensitized to birch components (PPA = 86%, NPA = 99%). The NPA was $\geq 91\%$ for all allergens, regardless of the cut-off used for the extract-based method (Table S3C and D). Similarly, agreement between component and extract IgE sensitization was lower for cat (kappa: 0.61 ± 0.04) than timothy (kappa: 0.81 ± 0.05) ($P = 0.002$) or birch (kappa: 0.89 ± 0.05) ($P < 0.001$).

Strong correlations were found between IgE antibody concentrations for allergen extracts and sum of IgE for different components of separate allergens: $r = 0.95$ for birch, $r = 0.96$ for timothy and $r = 0.83$ for cat (Fig. 1).

Table 2. Characteristics of subjects sensitized to timothy allergen extract and allergen components

Timothy	Extract— components—	extract+ components—	<i>P</i> value*	extract+ components+	<i>P</i> value*	<i>P</i> value [†]
<i>N</i>	350	23		86		
Levels of IgE against timothy extract (95% CI) (kU/L) [‡]	0.17	0.8 (0.6, 1.0)	<0.001	6.7 (5.1, 8.7)	<0.001	<0.001
Profilin subset (%)	0.3	0	0.8	12	<0.001	0.09
Polcalcin subset (%)	0.3	0	0.8	4.7	<0.001	0.3
Asthma (%)	13.1	17.4	0.56	51.2	<0.001	0.004
Rhinitis (%)	39.1	77.3	<0.001	78.8	<0.001	0.88
FeNO (ppb)	18.5 (17.1, 20.0)	21.7 (14.4, 32.6)	>0.99	27.3 (22.4, 33.3)	<0.001	0.71
Slope (units)	7.4 (7.1, 7.6)	7.2 (6.3, 8.2)	>0.99	6.2 (5.7, 6.8)	<0.001	0.60
S-ECP (mg/L)	7.8 (7.3, 8.4)	6.9 (5.2, 9.0)	>0.99	8.7 (7.5, 10.1)	0.6	0.45

*Against extract— components—.

[†]Against extract+ components—.[‡]Geometrical mean.

The clinical correlates of concordant/discordant patterns of IgE sensitization to extract and components for timothy and cat allergens

Subjects sensitized to both allergen extract and any component from timothy had more asthma ($P = 0.004$) than subjects positive to the extract but negative to components (Table 2). The prevalence of sensitization to profilin and polcalcin allergen components did not differ between subjects sensitized to only extract and subjects to both extract and any timothy component (Table 2), and the prevalence of asthma and rhinitis was not affected by profilin or polcalcin subset (data not shown).

Subjects sensitized to both extract and any component from cat had more asthma ($P = 0.032$), higher FeNO ($P = 0.008$) and S-ECP ($P = 0.030$), and more bronchial responsiveness ($P = 0.002$) than subjects positive to the extract but negative to components (Table 3).

Sensitization to both cat extract and any cat component, but not sensitization to only extract, was indepen-

dently associated with more bronchial responsiveness than in subjects not sensitized to cat, as showed in a multiple linear regression model adjusted for age, BMI, sex and smoking (Fig. 2a). Furthermore, sensitization to both cat extract and any cat component related to higher FeNO, as compared with sensitization to only extract (Fig. 2b). Similar analyses on sensitization to timothy did not turn out statistically significant (Fig. S4).

IgE sensitization patterns for cat and timothy in relation to new-onset rhinitis and asthma

Subjects without rhinitis ($n = 155$) or asthma ($n = 242$) at baseline (ECRHS II) were studied at the follow-up 12 years later (ECRHS III; Fig. 3). Subjects sensitized to both cat extract and components showed a higher prevalence of new-onset rhinitis and new-onset asthma compared with subjects sensitized only to the extract and with subjects without any cat allergen sensitization (Fig. 3). During the follow-up period, subjects

Table 3. Characteristics of subjects sensitized to cat allergen extract and allergen components

Cat	extract— components—	extract+ components—	<i>P</i> value *	extract+ components+	<i>P</i> value*	<i>P</i> value [†]
<i>N</i>	348	56		61		
Levels of IgE against cat extract (95% CI) (kU/L) [‡]	0.17	1.3 (1.0, 1.7)	<0.001	5.6 (4.3, 7.3)	<0.001	<0.001
Asthma (%)	11.5	37.5	<0.001	57.4	<0.001	0.032
Rhinitis (%)	40.7	67.9	<0.001	75.4	<0.001	0.38
FeNO (ppb)	17.9 (16.6, 19.2)	22.3 (17.6, 28.1)	0.13	33.6 (27.0, 41.8)	<0.001	0.008
Slope (units)	7.5 (7.2, 7.7)	6.8 (6.1, 7.7)	0.49	5.5 (5.0, 6.1)	<0.001	0.002
S-ECP (mg/L)	7.6 (7.1, 8.2)	7.4 (6.1, 8.8)	>0.99	10.2 (8.6, 12.1)	0.002	0.030

*Against extract— components—.

[†]Against extract+ components—.[‡]Geometrical mean.

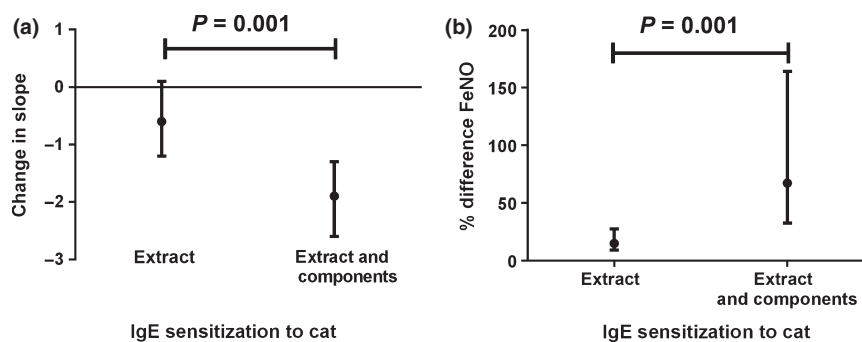


Fig. 2. Difference in bronchial responsiveness (lower values show more responsiveness, Panel a) and % difference of fraction of exhaled nitric oxide (FeNO) (Panel b) between subjects sensitized to cat extract or both extract and components vs. subjects not sensitized to cat. Results are from a multiple linear regression model and adjusted for age, BMI, sex, smoking at baseline. (Reference group are the subjects negative to both cat extract and any cat component.)

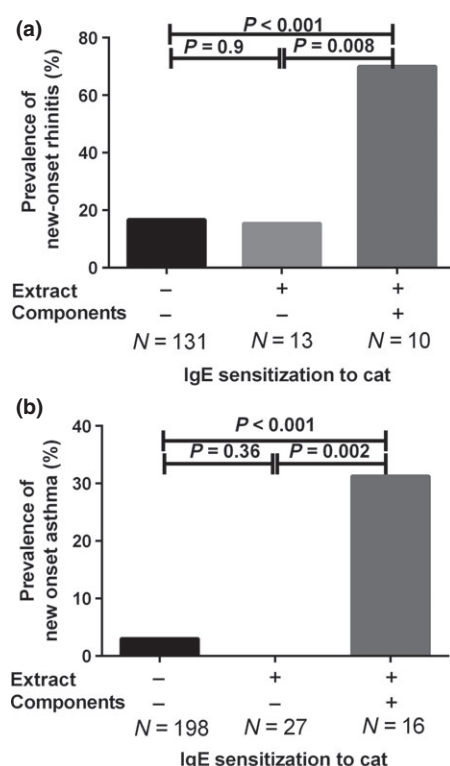


Fig. 3. Incidence of new-onset rhinitis (Panel a) among subjects without rhinitis at baseline ($n = 155$) and new-onset asthma (Panel b) among subjects without asthma at baseline ($n = 242$), in relation to the pattern of IgE sensitization to cat assessed with allergen extract and allergen components.

sensitized to both timothy extract and components had higher incidence of rhinitis ($P < 0.001$), but not of asthma ($P = 0.30$), than subjects without any timothy allergen sensitization (Fig. S5). Similar results were found for both cat and timothy when using a lower cut-off (≥ 0.1 ISU) for component sensitization (data not shown).

In multiple logistic regression models, new-onset rhinitis was associated with sensitization to cat compo-

nents, but not with sensitization to the extract (Fig. 4a). This association remained significant when analysing the additive value of sensitization to cat components in a model containing age, sex, BMI, smoking and asthma at baseline (Pseudo- R^2 increased from 13% to 20%, $P = 0.007$), while no additive value of sensitization to cat extract was found ($P = 0.15$). New-onset asthma was associated with sensitization both to cat extract and to cat components (Fig. 4b). Similarly, there was a consistent additive explanatory value of IgE sensitization to cat components for new-onset asthma (Pseudo- R^2 increased from 7% to 22%, $P = 0.005$), but this was not found for the extract ($P = 0.15$). New-onset rhinitis and asthma were significantly related to IgE sensitization to cat components but not to sensitization to cat extract if the models also included adjustment for specific IgE against cat extract (Fig. 4c,d). Sensitization to timothy allergen components was associated with new-onset rhinitis [OR 4.0 (1.03, 15.7)], but this was not found for timothy extract [OR 2.4 (0.6, 9.6)]. No association between new-onset of asthma and sensitization to timothy extract was found [OR 0.6 (0.1, 5.7)], and no subjects with sensitization to timothy components had new-onset asthma in the present study.

Relative importance of different cat and timothy components for airways inflammation and responsiveness and new-onset respiratory disease

An independent association was found between sensitization to Fel d 1 and Fel d 2 and FeNO ($P = 0.002$ and $P < 0.001$) and bronchial responsiveness ($P = 0.001$ and $P = 0.02$) when analysing independent effects of IgE sensitization to cat components in the same model (Table S4). Regarding new-onset respiratory disease, only trend towards an association between IgE sensitization to Fel d 1 and new-onset rhinitis ($P = 0.083$) was found (Table S4A). Similar

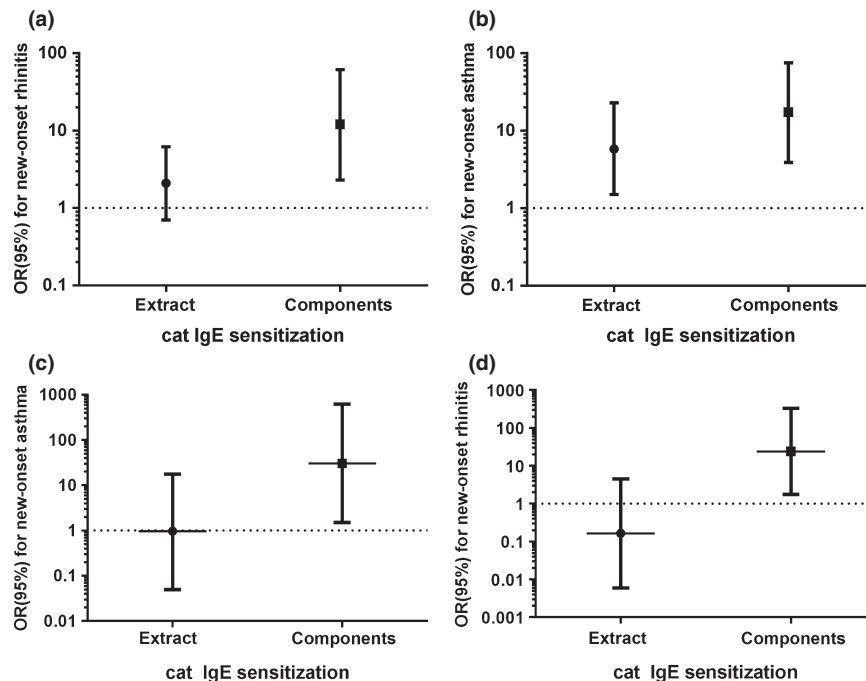


Fig. 4. Odds ratios for new-onset rhinitis[#] and new-onset asthma^{*} for subjects sensitized to cat allergens (Panels a, b, c[§], d[§]). [#]Adjusted for age, BMI, sex, smoking, asthma at baseline, ^{*}Adjusted for age, BMI, sex, smoking, rhinitis at baseline. [§]Adjusted even for degree of IgE sensitization to cat extract.

results were found for cut-off limit >0.1 ISU for cat components (Table S4B).

Regarding timothy components, only IgE sensitization to Phl p 1 was independently associated with FeNO ($P = 0.03$) and bronchial responsiveness ($P = 0.009$). Furthermore, IgE sensitization to Phl p 1 was associated with new-onset rhinitis ($P = 0.03$). (Table S4A). If a cut-off limit 0.1 ISU was used, the association between FeNO and Phl p 1 became weaker ($P = 0.09$) and the association between FeNO and Phl p 4 stronger ($P = 0.006$) (Table S4B).

Discussion

This study found a high overall correlation between the results of the extract-based and the component-based methods in measuring concentrations of IgE antibodies for cat, birch and timothy. Especially, a very strong negative per cent agreement was seen for all allergens. However, despite the high overall correlation, there was a substantial group of subjects who tested IgE-positive for cat and timothy extracts but negative for the corresponding components. Thus, the positive per cent agreement was less strong for these allergens, and this was especially pronounced for cat. Subjects with sensitization to both cat extract and components were characterized by higher FeNO and S-ECP, and more bronchial responsiveness than subjects with sensitiza-

tion only to cat extract. Furthermore, the individuals with sensitization to cat allergen components presented a higher likelihood of developing asthma and rhinitis during a 12-year follow-up period.

The high overall correlation between IgE sensitization to allergen extract and the corresponding components in this study is in agreement with previous studies on measurements of IgE towards single allergen components [15–18, 20]. Further, high concordance has been shown between the use of timothy extract and allergen components measured with multiplex technique [12, 13]. However, in our study, large variations in the positive per cent agreement between the methods were noted with the lowest PPA for cat. The PPAs for timothy and cat were lower than those presented by Bonini et al. [12] and Melioli et al. [13]. As these two studies and our study use the same version of the ISAC microarray, a possible explanation for this difference could be that our study is population-based, while the other two studies examined multi-sensitized subjects who probably had higher IgE antibody concentrations overall. In our study, the group with dissociation between IgE to extract and component become larger if total IgE < 50 kU/L than in subjects with total IgE $\geq$ 50 kU/L. Furthermore, as many allergic patients are co-sensitized to several domestic animals such as cat, dog or horse [18] suggesting strong comorbidity and/or prevalent cross-reactivity, a fact might explain some of

the dissociation between IgE sensitization to allergen extract and allergen components for cat. The greatest discordance was seen for comparisons between allergen extract and allergen components for cat. The subjects who were not sensitized to cat components on the ISAC chip had generally lower levels of IgE to cat extract. However, there was an overlap in IgE levels to cat between subjects sensitized and subjects not sensitized to cat components, and we still had discordant groups when raising the threshold for IgE sensitization to cat extract. It is possible that other allergen molecules as Fel d 7 who has about 60% of sequence identity with Can f 1 [35] could be the reason of cat sensitization according to extract tests without a genuine recognition. Cross-reacting carbohydrate determinants (CCD) can give positive results to extract-based tests for pollen, latex and venom allergens [36–38]; however, CCD reactivity could not explain the discordance in the present study in timothy sensitization due to its very low prevalence.

Participants who were positive to both cat extract and components had a higher prevalence of asthma, higher FeNO and more bronchial responsiveness compared with subjects sensitized only to the extract. It also seemed that assessment with cat components had a higher predictive value for the risk of developing asthma and rhinitis than analyses with the extracts. The reason for this is unclear, but it may relate to the fact that allergen extracts contain other allergen components not tested for, and that these components are of probably less clinical importance. Such allergen components for cat are Fel d 3 (cystatin) [39], Fel d 5w (cat IgA) [40, 41], Fel d 6 (cat IgM) [40, 41], Fel d 7 (cat IgG) [40, 41] and Fel d 8 (latherin) [35, 41]. Fel d 5w is detected in nearly 40% of cat sensitized individuals [40] and occurs often in subjects presenting with food-related urticarial or anaphylactic reactions [42]. Fel d 7 is present in about 38% of subjects with cat allergy and presents cross-reactivity with dog dander extracts [35]. Sensitization to certain allergen components might be more clinically relevant as timothy component Phl p 1 was independently associated with new-onset rhinitis, higher FeNO and bronchial responsiveness, while cat components Fel d 1 and Fel d 2 were associated with bronchial responsiveness and FeNO. A previous study has also shown the association of Phl p 1 with new-onset rhinitis [25], but no other study examined the association between timothy or cat components and new-onset rhinitis, FeNO or bronchial responsiveness. Another explanation may be that analysis of allergen components by multiplex technique is less sensitive [13], meaning that we will only detect individuals with higher IgE antibody concentrations, with a concurrent increased risk of developing airway inflammation and respiratory symptoms. However, this theory fails to

explain why we could still find a significant proportion of subjects without sensitization to cat allergen components even when looking at subjects with IgE levels >1 kU/L towards the extract.

IgE sensitization to cat and timothy allergen components had additive predictive value for new-onset asthma and rhinitis compared with a confirmed sensitization to the corresponding extracts. Earlier studies on the predictive value of extract-based IgE measurements for the onset of allergic diseases in adults have shown that IgE to grass and birch pollen allergens were related to new-onset rhinitis [43, 44], and IgE to cat was related to new-onset asthma [45]. To our knowledge, only one study has examined the prognostic value of IgE sensitization to allergen components. This was performed in children and found a relation between timothy components and new-onset rhinitis [25].

The present study is, to our knowledge, the first to examine the prognostic value of IgE sensitization to allergen components in relation to new-onset rhinitis or asthma in adults. A strength of the study is the long follow-up period of 12 years, which provided us with a sufficient number of subjects with incident respiratory disease. A weakness of the study may be that the definition of allergic disease was questionnaire-based. Further, caution should be exercised in making inferences about how results based on multiplex technique can be applied to analyses of IgE to single components. Another limitation is that the version of the ISAC chip used in that study is no longer available, and thus, data presented here might be difficult to be replicated in the future.

In conclusion, measurement of sensitization to cat allergen components using multiplex technique appeared to be superior to measurement of IgE sensitization to the corresponding extract, as regards the association with asthma morbidity in terms of airway inflammation and responsiveness. In longitudinal analyses, IgE sensitization to cat and timothy components had stronger prognostic values for new-onset rhinitis and asthma, compared with traditional extract-based methods.

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Conflict of interest

MB is an employee of Thermo Fisher Scientific.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. The studied population.

Figure S2. Venn diagram of subjects sensitized to timothy components (only the 3 most common) (a) and cat components (b).

Figure S3. Aligned dot plot of IgE levels against cat extract (kU/L) for subjects sensitized to both cat extract and cat components vs. subjects sensitized to cat extract but not to cat allergen components.

Figure S4. Difference in bronchial responsiveness (slope) (more negative values show more responsiveness, Panel a) and % difference of FeNO (Panel b) between subjects sensitized to timothy extract or extract and component vs. subjects not sensitized to timothy.

Figure S5. Incidence of new onset rhinitis (Panel a) at ECRHS II ($n = 155$) and asthma among subjects without asthma (Panel b) at ECRHS II ($n = 242$) in relation to timothy sensitization pattern assessed with allergen extract and allergen components.

Table S1a. Characteristics and type of sensitization in subjects (who were not followed at follow up) divided into groups based on their respiratory disease status at baseline (cut-off limit for allergen components 0.30 ISU and for allergen extract 0.35 kU/L).

Table S1b. Characteristics and type of sensitization in subjects divided into groups based on their respiratory

disease status at baseline (cut-off limit for allergen components 0.1 ISU).

Table S2. Prevalence type of sensitization (allergen extract with cut-off 1 kU/L) in subjects based on respiratory disease status at baseline.

Table S3. Comparison of allergen components vs. allergen extract-based methods for total IgE <50 kU/L (a) and total IgE ≥ 50 kU/L (b) and for allergen extract with cut-off level 1 kU/L (c) or 3.5 kU/L (d) and for allergen components with cut-off level 0.1 ISU (e).

Table S4a. Independent association between FeNO (significant when it does not contain value zero), slope (where lower values indicate more bronchial responsiveness, and it is significant when it does not contain value one) new-onset rhinitis# and new-onset asthma* and cat or timothy components in the same model #Adjusted for age, BMI, sex, smoking, asthma at baseline, *Adjusted for age, BMI, sex, smoking, rhinitis at baseline. §Adjusted for age, BMI, sex, smoking.

Table S4b. Independent association between FeNO (significant when it does not contain value zero), slope (where lower values indicate more bronchial responsiveness, and it is significant when it does not contain value one) new-onset rhinitis# and new-onset asthma* and cat or timothy components in the same model (cut off limit 0.1 ISU) #Adjusted for age, BMI, sex, smoking, asthma at baseline, *Adjusted for age, BMI, sex, smoking, rhinitis at baseline. §Adjusted for age, BMI, sex, smoking.