IgE sensitization against food allergens - Natural history, relation to airway inflammation and asthma

ANTONIOS PATELIS
Abstract

Background: According to recent studies in children, IgE sensitization not only against perennial allergens, but also against food allergens, is related to asthma risk and increased airway inflammation. During the last decade, a new technique for IgE determination based on allergen components has become available, but its use in epidemiological studies has been limited.

Aims: To investigate the relationship between the pattern of IgE sensitization to allergen components and the prevalence of asthma, airway inflammation and hyperresponsiveness in a population-based setting. To examine the relationship of IgE sensitization to allergen extract, and airway inflammation, airway hyperresponsiveness and blood eosinophilia in asthmatics. To examine the natural history of IgE sensitization to food allergens in adults. To compare extract-based and component-based IgE measurements in relation with new-onset respiratory disease and airway inflammation and hyperresponsiveness.

Methods: The present thesis is based on cross-sectional and longitudinal analyses of the adult, the population-based study ECRHS (European Community Health Survey) and a cross-sectional, observational study of young subjects with asthma. IgE sensitization was examined by means of both extract-based and component-based tests. Airway inflammation was assessed by exhaled NO and airway hyperresponsiveness with methacholine test.

Results: IgE sensitization to food allergens independently related to increased airway inflammation in both a population-based study and a study of asthmatics. Furthermore, a relation was found with increased blood eosinophils in asthmatics. The decrease in prevalence of IgE sensitization against food allergens during a 9-year follow-up was larger than the decrease of aeroallergens. Subjects with IgE sensitization to both cat extract and components showed more frequent airway inflammation, greater bronchial responsiveness and higher likelihood of developing asthma and rhinitis than subjects with IgE sensitization only to cat extract.

Conclusions: The presence of IgE antibodies against food allergens was independently associated with airborne and systemic inflammation. Both aeroallergens and food allergens should be examined in order to understand the signaling of local and systemic inflammation in asthma. Prevalence of IgE sensitization to food decreased in adults to a larger extent than IgE sensitization against aeroallergens. Measurement of IgE sensitization to cat allergen components appears to have a higher clinical value than extract-based measurement.

Keywords: allergen components, extract-based, exhaled NO, airway hyperresponsiveness, blood eosinophil count, IgE sensitization, food hypersensitivity, food allergens, perennial allergens, natural history, markers of systemic inflammation, markers of local inflammation

Antonios Patelis, Department of Medical Sciences, Akademiska sjukhuset, Uppsala University, SE-75185 Uppsala, Sweden.

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When you feel that you want to quit after a bad result think that nobody managed to walk without falling, nobody managed to run without stopping to get some breaths.
List of papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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Discussion

Asthma, degree of inflammation and airway hyperresponsiveness in relation to pattern of IgE sensitization in a general population and in a cohort of asthmatics

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Change in the prevalence of IgE sensitization to food allergens

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Study limitations

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Conclusions

Clinical implications

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References
Abbreviations

B-Eos  Blood eosinophil count
BMI    Body mass index
ECRHS  European Community Respiratory Health Study
FeNO   Fraction of exhaled nitric oxide
FEV₁   Forced expiratory volume in one second
FVC    Forced vital capacity
IgE    Immunoglobulin E
IL     interleukin
iNOS   inducible NO synthase
ISAC   Immuno-solid-phase allergen chip
ISU    ISAC standardized units
MIDAS  Minimally-invasive diagnostics for asthma allergic diseases
PD₂₀ FEV₁ Cumulative dose of methacholine causing a 20 % reduction in FEV₁
sIgE   specific IgE antibodies in serum
Introduction

IgE sensitization and asthma

Asthma is a significant health problem affecting 300 million people of all ages worldwide (1). The prevalence of asthma is increasing in most countries, especially among children. Consequences of asthma can be heavy for the individual due to limitation of daily activities and poorer quality of life, but also for society due to healthcare costs. In some cases, uncontrolled asthma without proper treatment can be fatal (1). According to a UK study, deaths because of anaphylaxis affected mostly patients treated for asthma, and in most of these individuals, asthma was poorly controlled (2). Our understanding of asthma has progressed greatly in the last decades and modern treatment can be effective, allowing asthmatics to lead normal lives (3). However, asthma control – the goal of asthma treatment – is not achieved in more than 50 % of patients (4, 5).

The prevalence of allergic diseases is also increasing in many countries and it is estimated that 25 % of the population worldwide suffers from allergies (6). Allergic sensitization is a known risk factor for asthma (7) and sensitization to multiple allergens is associated with a high prevalence (8) and severity of asthma (9). Synthesis of IgE against allergens is a prerequisite for developing allergic diseases such as allergic rhinitis and asthma (10), although many sensitized individuals do not develop symptoms (11). Moreover, there are subjects who have non-allergic asthma (3). The prevalence of atopy in asthmatics approaches 80 % in a national survey in the United States and could be the causative factor in over 50 % of asthma cases (7). The European Community Respiratory Health Study (ECRHS) found a prevalence of atopy of 70 % in asthmatics (12) and an overall attributable fraction of asthma symptoms caused by atopy of 30 % (13).

IgE sensitization and airway inflammation

One of the key features in asthma is chronic airway inflammation, in which many cells and cellular elements play a role (1). This chronic inflammation in the airway mucosa accounts to a large extent for the symptoms that an asthmatic patient experiences, as the accompanying airway hyperresponsiveness results in repeated attacks of wheezing, breathlessness, chest tight-
ness and coughing, particularly at night (14). Exhaled nitric oxide (NO) originates from the bronchial epithelium and can be used as a marker of airway inflammation (15). A marked upregulation of inducible NO synthase (iNOS) in the epithelium of the airways of asthmatics through the signaling pathway of interleukins IL-4 and IL-13 seems to be the main cause for increased NO levels (16-18). By contrast, patients with non-allergic asthma have lower levels of exhaled NO (19, 20) often within normal range. However, these levels appear to be higher than in their non-allergic healthy counterparts (21). Increased exhaled NO levels have been associated with allergic sensitization and the degree of IgE sensitization is related to exhaled NO (22, 23). Exhaled NO is not a marker of atopy per se (24), but rather a result of the subclinical airway inflammation caused by allergen exposure through either environmental allergens (25) or under-controlled challenge (18). Finally, having increased NO levels is a prognostic factor for the development of respiratory symptoms in the future, in both children (26) and adults (27).

IgE sensitization and airway hyperresponsiveness

Airway hyperresponsiveness to specific and non-specific stimuli (1) is a consistent and defining clinical feature of asthma. Airway hyperresponsiveness consists of a variable and a persistent component (28, 29) and can be measured as the responsiveness to direct (histamine, methacholine) and indirect (mannitol, adenosine monophosphate, exercise, cold air, hyperventilation) stimuli (30, 31). According to previous studies (28) the persistent component is observed frequently, but not always, and is related to the chronicity of the disease. Chronic effects of airway inflammation lead to airway remodeling which includes increased contractility of smooth muscles, increased wall thickness and reduced airway caliber (28). Furthermore, other studies (32, 33) indicate that the variable component reflects airway inflammation and is related to allergic sensitization. There is persuasive evidence (28) that allergen-induced reactions in the airways, such as airway hyperresponsiveness and eosinophilic inflammation, are IgE-dependent. Additionally, the monoclonal anti-(Fc)-IgE omalizumab inhibits these asthmatic responses as well as allergen-induced airway hyperresponsiveness and eosinophilia (34, 35). Airway hyperresponsiveness is associated not only with the presence, but also with the degree of atopic sensitization (36).

IgE sensitization and blood eosinophil count

Peripheral blood eosinophilia is also recognized as a feature of allergic sensitization (37-39) and asthma (40). Regardless of the clinical history of respiratory symptoms, exposure to allergens is sufficient for increased eosinophil
counts in sensitized subjects (37). Blood eosinophilia is primarily triggered by IL-5 (41), but can be also driven by other mechanisms, such as rhinovirus infections (42) or chronic rhinosinusitis (43). Eosinophils are major effector cells in asthma (40), although some patients, especially those with non-allergic asthma, have a neutrophilic or paucigranulocytic profile (3). The previous theory, that systemic eosinophilic inflammation originates in a spillover from the inflamed airways, is challenged by a new one suggesting that they are two independent inflammatory components (24). This notion is supported by the finding that airway inflammation and blood eosinophilia correlate only moderately (44). Furthermore, local anti-inflammatory drugs, such as inhaled steroids, do not affect peripheral eosinophilia (45) as much as systemic anti-inflammatory drugs like antileukotrienes (46, 47). The systemic eosinophilic inflammation, measured by blood eosinophil count (B-Eos), is of importance for asthma, as the number of eosinophils in peripheral blood and in bronchial lavage from subjects with asthma is associated with disease severity (48) and asthma-related visits to emergency departments (49).

Importance of type of IgE sensitization

IgE sensitization against aeroallergens and respiratory disease

IgE sensitization is associated with developing asthma and other atopic diseases, but not in an absolute manner. Rather, certain patterns (50) and degrees (51) of IgE sensitization are linked to a higher probability of new-onset allergic disease. Sensitization against perennial aeroallergens is the type of IgE sensitization that seems to have the higher clinical impact on asthma and its clinical features. Specifically, asthma is strongly associated with elevated IgE and sensitization to indoor perennial allergens, notably those derived from cat (52) and mite (53). Sensitization to perennial but not seasonal allergens is reported to be the main determinant of high exhaled NO levels (22). Sensitization to indoor allergens (mite or cat) has the strongest association with airway hyperresponsiveness in ECRHS (54) and other surveys (53-56). Having IgE antibodies against perennial allergens is related to new-onset asthma (57, 58), whereas IgE sensitization against seasonal (pollen) allergens has been shown to be more related to rhinitis than asthma in both cross-sectional (59) and longitudinal studies (60). There are also geographical differences concerning which allergic profile is most clinically relevant for asthma and airway inflammation. IgE sensitization against mite allergens is more important in Southern Europe (61, 62) while IgE sensitization against furry animal (cat and dog) is more important in Northern Europe (8, 63).
IgE sensitization against food allergens and adverse food reactions

It is important to point out the difference between IgE sensitization towards food allergens, IgE-mediated food allergy and food hypersensitivity. The majority of subjects sensitized to food allergens do not present clinical IgE-mediated food allergy; only about one tenth of those sensitized to a food allergen develop symptoms of food allergy (64). It is also a fact that most adverse reactions after food ingestion are not IgE-mediated (65). Therefore, they cannot be classified as IgE-mediated food allergy, but should rather be characterized as food hypersensitivity (66). This term covers all non-toxic food reactions which are further divided in immune-mediated (IgE- and non-IgE-mediated food allergy) and non-immune-mediated (10). Food hypersensitivity is also common. Previous population studies in the 1990s have estimated the prevalence of self-reported adverse reactions after food consumption to 12-20 % in adults (67-69). Food hypersensitivity reactions are usually non-fatal, but have important impact on quality of life even if they are just perceived reactions and not physician-diagnosed (70). Further studies in adults are needed as there are limited data on the prevalence and distribution of IgE sensitization to food allergens in relation to food hypersensitivity.

In one study it was found that 15 % to 20 % of the population has IgE antibodies against at least one food allergenic source (64). In the same cross-sectional study (64), prevalence of IgE sensitization towards food allergens was highest in children, at 28 %, and decreased with age to 13 % in adults older than 60 years old. The majority of allergic reactions to food allergens begin in childhood and few treatment alternatives can influence their natural history (71). Existing longitudinal studies (72, 73) about the natural course of IgE sensitization to food are few and most cover only childhood, where prevalence remains stable during the first 5-6 years of life and then begins to decline. Unfortunately, data about sensitization in adults are scarce. The prevalence of food allergy has been found to be 6 % in children (74) and 4 % in adults (75). Childhood food allergies are usually outgrown (76-79), with the exception of peanut allergies, which usually persist (80, 81).

IgE sensitization against food allergens and respiratory disease

It is important to map IgE sensitization against food allergens. According to recent studies in children (82, 83), children sensitized to food allergens have increased levels of FeNO and have an increased risk for asthma. Moreover, IgE sensitization to food is highly prevalent in adults (39) as well as children (84) with asthma, and is associated with increased use of healthcare and asthma medication (84). Furthermore, IgE-mediated food allergy can act as a trigger for asthma symptoms (3) and may be an underestimated risk factor for severe asthma (85). In patients with diagnosed food-induced anaphylaxis,
the severe allergic reaction may include a life-threatening asthma attack (86). It is important to define predisposing factors associated with worse asthma control, as asthma is often not optimally controlled, despite existing therapies (4, 87, 88). Moreover, recognizing risk factors for new-onset asthma and modifying them may reduce the burden of disease in the future (89).

Methods for examining IgE sensitization

Extract-based tests

The most important step in the diagnostic algorithm of allergic diseases is a thorough clinical case history. Traditionally IgE sensitization has been examined with skin prick tests (SPT) or in vitro specific tests (sIgE), when patient history has suggested sensitization against an allergenic source (90). These IgE antibody tests are based on crude extracts consisting of allergenic and non-allergenic molecules from that source (91). While the quality of the extracts has varied in the past (90, 92, 93), advances in characterization and standardization have improved quality (94, 95). Skin prick test is a cheaper method with high sensitivity that allows immediate reading, while blood tests constitute an automatic quantitative method appropriate for high sensitivity monitoring of sensitization (91).

Component-based tests

To supplement the traditional extract-based test, component-resolved IgE antibody determination, based on microarray techniques, was recently introduced into the field of clinical Allergology (96). The utilization of advances in DNA technology allowed the characterization and cloning of various allergenic molecules in order to determine important components of various allergic diseases (97-100). The availability of allergenic components in the last decades exploded and created a need for systematic allergen nomenclature. The World Health Organization and International Union of Immunological Species Allergen Nomenclature Subcommittee were charged with establishing and maintaining the systematic nomenclature and a detailed database of characterized allergenic proteins. Allergenic molecules are named using their Latin family name (genus and species). For example, the abbreviation Fel d is used for allergenic components from Felis domesticus (cat). A number is added to the name to distinguish the various components from the same species (e.g., Fel d 1, Fel d 2, etc.). The numbers are given to allergens in order of identification.

The various allergenic components are grouped into protein families, based on their structure and biological function (101). Some protein families, such as storage proteins in peanuts (e.g., Ara h 2) and hazelnuts (e.g., Cor a
9), more frequently trigger severe allergic reactions such as anaphylaxis, while other allergenic molecules are considered more innocent as they associate to sensitization without severe clinical symptoms. Components belonging to the same family have similar antibody binding sites (epitopes) and the same IgE antibody can bind allergens from different allergen sources. For example, Bet v 1, the major birch pollen allergen, shows high homology with other members of pathogenesis-related (PR)-10 protein family such as Ara h 8, a peanut allergen, or Cor a 1, a hazelnut allergen. IgE antibodies against birch cross-react with peanut and hazelnut causing allergic symptoms usually in form of oral allergy syndrome. These cross-reactive allergen components can explain many sensitizations and clinical symptoms from related allergenic sources (102-105).

In contrast to cross-reactive components, there are also components specific for a certain allergenic source. This is an important utility of component-based diagnostics as it identifies markers of primary sensitization and increases the accuracy of diagnosis (91). Component-based analysis facilitates a better understanding of primary sensitizations (106, 107) and eases a risk assessment for future severe allergic reactions (102, 108). This can improve the selection of patients appropriate for immunotherapy and the effect of targeted allergen avoidance or the need to proceed with food challenges (102). However, the geographical differences in distribution and prevalence of various sensitization patterns and disease expression should be taken in consideration in the evaluation of the information obtained with this method.

Component-resolved IgE antibody determination is a new semi-quantitative technique, still under validation and it is considered to be a third line tool after clinical history and extract-based tests (91). This new technology can also be useful as a tool for evaluation of the epidemiology of IgE sensitization in the future (109).

### Extract-based test vs. component-based tests

Several child cohort studies (110-112) have examined sensitization using both extract-based tests and allergen components, though none of these studies included a direct comparison. IgE testing against peanut components had a high diagnostic accuracy in children with peanut allergy verified through food challenges. Only a few cross-sectional surveys (113-115) have examined the performance of IgE determination using allergen components in relation to IgE determination using extracts, or examined the relation of IgE determination using allergen components to clinical symptoms (116). Reports generally indicate a high degree of agreement between single and multiplex measurements of IgE to allergen components (113), as well as between measurements using allergen extract and the respective components (117-122). However, differences have been reported for some allergens (among them cat, timothy and *Dermatophagoides pteronyssinus*) (114, 115),
with a fraction of subjects showing sensitization in extract-based tests, but not in tests using allergen components. This results in a high negative percent agreement and a lower positive percent agreement, despite the overall high concordance between the two types of IgE measurements. 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.
Aims

1) to investigate the relationship between the pattern of IgE sensitization, obtained using the microarray technique, and the prevalence of asthma, levels of exhaled NO and airway hyperresponsiveness in a population-based setting.

2) to investigate the IgE sensitization profile in asthmatics and examine the association between this profile and exhaled nitric oxide, blood eosinophil count and airway responsiveness to methacholine.

3) to examine the natural course of food hypersensitivity symptoms and IgE sensitization to food allergens in adults and to investigate the risk factors for new-onset food hypersensitivity and sensitization.

4) 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 hyperresponsiveness.
Material and methods

Population
The populations of Papers I, III and IV were based on the European Community Respiratory Health Survey (ECRHS) I (1991-92), II (2000-01) and III (2010-2012) (123), an international multicenter study of asthma and allergy (ECRHS) and its follow-ups after ~10 and ~20 years respectively. The details of the designs of ECRHS I, II and III have been published (123). Each participant was sent a brief questionnaire (Stage 1) and among those who responded, a random sample was invited to undergo a more detailed clinical examination (Stage 2). A "symptomatic" sample consisting of additional subjects who in Stage 1 reported symptoms of asthma attacks, using asthma medication or waking with shortness of breath was also studied. In ECRHS II, subjects who had participated in Stage 2 were invited to participate in a follow-up study. Subjects answered a standardized questionnaire administered by trained interviewers and underwent lung function tests and blood tests. In the Uppsala study center, 679 subjects were reinvestigated in ECRHS II. ECRHS III encompassed 302 subjects who had participated in ECRHS II. Rhinitis and asthma symptoms were questionnaire-assessed again in ECRHS III.

Paper I included 467 people from the Uppsala study center in ECRHS II, who were examined with ImmunoCAP ISAC. Of these people, 96 were diagnosed with symptomatic asthma. Methacholine challenge test results were available from 362 subjects and exhaled NO from 288.

Paper III comprised 2,307 individuals from Sweden and Iceland who answered questions about food hypersensitivity symptoms in both ECRHS I and II. In a subgroup of these ECRHS participants from the random sample (n = 807), for whom there were measurements of IgE to food allergens at ECRHS I (39) (124), these measurements were performed again in the ECRHS II. (Figure 1)
Paper IV was based on 451 subjects from Uppsala center, who participated in the ECRHS II, which was a 9-year follow-up of the first ECRHS (125) and 302 subjects from the ECRHS III, which was a 10-year follow-up of ECRHS II.

This study focused on 302 people who were examined with both allergen extracts (ImmunoCAP IgE) and allergen components (ImmunoCAP ISAC) at baseline and followed up 10 years later with a questionnaire about respiratory symptoms and disease in the ECRHS III (Figure 2).
Figure 2. Flow chart explaining the studied population of Paper IV.

Paper II was based on subjects who participated in a project run within the framework of an industry-academy collaboration on Minimally-Invasive Diagnostics for Asthma and allergic diseases (MIDAS). Subjects were recruited from both primary and specialist care in Uppsala, Sweden. A total of 408 children and young adults (10-34 years) with physician-diagnosed asthma (according to their medical records) and daily treatment with inhaled corticosteroids and/or oral antileukotrienes during at least three months of the year before the study were included in Paper II. Further, 118 non-asthmatic controls were randomly chosen from the population registry and also included in the paper.

Questionnaires

The main questionnaire used in ECRHS I, II and III (http://www.ecrhs.org (125)) gathered information about respiratory symptoms, respiratory disease (asthma and rhinitis), food hypersensitivity and smoking history.
Definition of asthma
In Papers I, III and IV, 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: nocturnal chest tightness, attack of shortness of breath, chest wheezing or whistling (126).

In Paper II, subjects participated in detailed interviews containing questions regarding symptoms of asthma, rhinitis and sinusitis as well as medication use.

The degree of asthma control was assessed with the Asthma Control Test (ACT) (127), which is a questionnaire consisting of 5 questions, each with a 5-point scale. The total ACT score is between 5 and 25, with a lower score indicating poorer control of asthma. An ACT score $\geq 20$ reflects well-controlled asthma (127).

Definition of rhinitis
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?” (128).

Definition of food hypersensitivity
An individual was considered to have food hypersensitivity (10) if he/she answered yes to the following questions “Have you ever had an illness or trouble caused by eating a particular food or foods?” and if so, “Have you nearly always had the same illness or trouble after eating this type of food?” Respondents were then asked to list the food(s) and symptoms (67).

Presence and degree of IgE sensitization
In Paper I, the presence of IgE antibodies was examined using microarray chip technology (ImmunoCAP ISAC; Phadia/Thermo Fischer Scientific, Uppsala, Sweden) (129) (130) (Table 1). Sensitization is given in ISU units as a semi-quantitative estimate of specific IgE antibody titer. Subjects were considered non-IgE-sensitized if the signal was non-measurable or very low (< 0.3 ISU). The IgE antibody levels were added together (sum IgE) (22) in order to get an assessment of the overall levels of IgE sensitization in four allergen categories: food allergens, seasonal allergens (pollen), perennial allergens (animal, mite, mold), and other allergens.
Table 1. List of available allergen components examined with ISAC chip used in papers I and IV.

<table>
<thead>
<tr>
<th>Foods from plant origin</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any</td>
<td></td>
</tr>
<tr>
<td>Brazil nut</td>
<td>nBer e 1</td>
</tr>
<tr>
<td>Sesame</td>
<td>nSes i 1</td>
</tr>
<tr>
<td>Peanut</td>
<td>nAra h 1</td>
</tr>
<tr>
<td></td>
<td>nAra h 2</td>
</tr>
<tr>
<td></td>
<td>nAra h 3</td>
</tr>
<tr>
<td></td>
<td>rAra h 8</td>
</tr>
<tr>
<td>Hazelnut</td>
<td>rCor a 1,0401</td>
</tr>
<tr>
<td>Apple</td>
<td>rMal d 1</td>
</tr>
<tr>
<td>Peach</td>
<td>rPru p 1</td>
</tr>
<tr>
<td></td>
<td>nPru p 3</td>
</tr>
<tr>
<td>Soybean</td>
<td>rGly m 4</td>
</tr>
<tr>
<td>Celery</td>
<td>rApi g 1</td>
</tr>
<tr>
<td>Carrot</td>
<td>rDau c 1</td>
</tr>
<tr>
<td>Kiwi</td>
<td>Act d 1</td>
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<tr>
<td></td>
<td>Act d 2</td>
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<td></td>
<td>Act d 5</td>
</tr>
<tr>
<td></td>
<td>Act d 8</td>
</tr>
<tr>
<td>Cashew</td>
<td>Ana o 2</td>
</tr>
<tr>
<td>Hazelnut</td>
<td>Cor a 8</td>
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<tr>
<td></td>
<td>Cor a 9</td>
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<tr>
<td>Soy</td>
<td>Gly m 5</td>
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<td></td>
<td>Gly m 6</td>
</tr>
<tr>
<td>Wheat</td>
<td>Gliadin</td>
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<td>Tri a 19</td>
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<td>Tri a 18</td>
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<td>Tri a aA_TI</td>
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<tr>
<td>Foods from animals</td>
<td>Any</td>
</tr>
<tr>
<td>Egg white</td>
<td>nGal d 2</td>
</tr>
<tr>
<td>Milk</td>
<td>nBos d 4</td>
</tr>
<tr>
<td></td>
<td>nBos d 5</td>
</tr>
<tr>
<td></td>
<td>nBos d 8</td>
</tr>
<tr>
<td></td>
<td>nBos d lactoferrin</td>
</tr>
<tr>
<td>Carp</td>
<td>rCyp c 1</td>
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<tr>
<td>Cod</td>
<td>rGad c 1</td>
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<tr>
<td>Shrimp</td>
<td>rPen a 1</td>
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<td>nPen i 1</td>
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<tr>
<td></td>
<td>nPen m 1</td>
</tr>
<tr>
<td>Egg white</td>
<td>nGal d 1</td>
</tr>
<tr>
<td></td>
<td>nGal d 3</td>
</tr>
<tr>
<td>Egg yolk/chicken</td>
<td>nGal d 5</td>
</tr>
<tr>
<td>Grass pollen</td>
<td><em>Any</em></td>
</tr>
<tr>
<td>Bermuda</td>
<td>nCyn d 1</td>
</tr>
<tr>
<td>Timothy</td>
<td>rPhl p 1</td>
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<td></td>
<td>rPhl p 2</td>
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<td>nPhl p 4</td>
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<td>rPhl p 12</td>
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<tr>
<td>Tree pollen</td>
<td><em>Any</em></td>
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<tr>
<td>Olive</td>
<td>nOle e 1</td>
</tr>
<tr>
<td></td>
<td>nOle e 2</td>
</tr>
<tr>
<td>Plane tree</td>
<td>rPla a 1</td>
</tr>
<tr>
<td></td>
<td>rPla a 2</td>
</tr>
<tr>
<td>Cypress</td>
<td>nCup a 1</td>
</tr>
<tr>
<td>Birch</td>
<td>rBet v 1</td>
</tr>
<tr>
<td></td>
<td>rBet v 2</td>
</tr>
<tr>
<td></td>
<td>rBet v 4</td>
</tr>
<tr>
<td>Alder</td>
<td>rAln g 1</td>
</tr>
<tr>
<td>Hazel</td>
<td>rCor a 1,0101</td>
</tr>
<tr>
<td>Japanese cedar</td>
<td>Cry j 1</td>
</tr>
<tr>
<td>Weed pollen</td>
<td><em>Any</em></td>
</tr>
<tr>
<td>Ragweed</td>
<td>nAmb a 1</td>
</tr>
<tr>
<td>Mugwort</td>
<td>nArt v 1</td>
</tr>
<tr>
<td></td>
<td>nArt v 3</td>
</tr>
<tr>
<td>Wall pellitory</td>
<td>Par j 2</td>
</tr>
<tr>
<td>Saltwort</td>
<td>Sal k 1</td>
</tr>
<tr>
<td>Animal</td>
<td><em>Any</em></td>
</tr>
<tr>
<td>Cat</td>
<td>rFel d 1</td>
</tr>
<tr>
<td></td>
<td>nFel d 2</td>
</tr>
<tr>
<td></td>
<td>rFel d 4</td>
</tr>
<tr>
<td>Dog</td>
<td>rCan f 1</td>
</tr>
<tr>
<td></td>
<td>rCan f 2</td>
</tr>
<tr>
<td>Category</td>
<td>Subcategory</td>
</tr>
<tr>
<td>----------</td>
<td>-------------</td>
</tr>
<tr>
<td>Mite</td>
<td>House dust mite</td>
</tr>
<tr>
<td>Mite</td>
<td>Storage mite</td>
</tr>
<tr>
<td>Mold</td>
<td>Alternaria</td>
</tr>
<tr>
<td>Mold</td>
<td>Aspergillus</td>
</tr>
<tr>
<td>Mold</td>
<td>Cladosporium</td>
</tr>
<tr>
<td>Cockroach</td>
<td></td>
</tr>
<tr>
<td>Latex</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>Mercury</td>
</tr>
<tr>
<td>Other</td>
<td>Bromelin</td>
</tr>
<tr>
<td>Other</td>
<td>Bee</td>
</tr>
<tr>
<td>Other</td>
<td>Anisakis</td>
</tr>
</tbody>
</table>
In Paper II, IgE against a mix of food allergens (fx5), hazelnut, shrimp, peach, a mix of aeroallergens (Phadiatop) and *Alternaria alternata* were measured in all subjects. Subjects with titers of $\geq 0.35$ kU/L against the aeroallergen or food mix were further characterized regarding IgE-antibody levels against each individual allergen in the respective panels: egg white, milk, cod fish, wheat, peanut, and soybean for the food allergen panel and cat, dog, horse, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Cladosporium herbarum*, birch, timothy grass, and mugwort for the aeroallergen panel. All measurements of IgE were done with the ImmunoCAP system (Immunodiagnostics, Thermo Fisher Scientific, Uppsala, Sweden). The results are presented in kU/L and values of IgE $\geq 0.35$ kU/L for an individual allergen defined a subject as being sensitized to that allergen (131). Subjects with IgE-antibody levels $< 0.35$ kU/L were given an arbitrary value of 0.17 kU/L. The IgE antibody levels were added together (sum IgE) (22), to get an assessment of the overall levels of IgE sensitization in five different allergen categories: Mite (*D. pteronyssinus*, *D. farinae*), Mold (*Cladosporium herbarum*, *Alternaria alternata/Alternaria tenuis*), Furry animal (cat, horse, dog), Pollen (timothy, birch, mugwort) and Food (wheat, peanut, soy, shrimp, egg, milk, fish) (Table 2).

**Table 2.** List of allergens examined with ImmunoCAP in Papers II, III and IV (*only in Paper II*).

<table>
<thead>
<tr>
<th>Aeroallergens</th>
<th>Mite</th>
<th><em>D. pteronyssinus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>D. farinae</em></td>
</tr>
</tbody>
</table>
| Mold                | *Cladosporium herbarum*
|                     | *Alternaria alternata/Alternaria tenuis* |
| Furry animal        | Cat                   |
|                     | Horse                 |
|                     | Dog                   |
| Pollen              | Timothy               |
|                     | Birch                 |
|                     | Mugwort               |
| Food                | Plant origin          |
|                     | Wheat                 |
|                     | Peanut                |
|                     | Soy                   |
|                     | Hazelnut *            |
|                     | Peach *               |
| Animal origin       | Shrimp *              |
|                     | Egg                   |
|                     | Milk                  |
|                     | Fish                  |

We chose not to include hazelnut and peach when evaluating the results in the group of food allergens due to the large extent of cross-reactivity in
birch-sensitized subjects with hazelnut (94 %) and peach (73 %). In order to adjust for a potential cross-reactivity between horse and cat IgE (132), we have performed additional analyses after removing horse-sensitized subjects from the group sensitized to furry animals.

In Paper III, venous blood samples were drawn and frozen at -20 °C for measurement of total serum IgE (total IgE) and serum IgE antibodies to cat, D. pteronyssinus, grass, birch, and Cladosporium, as part of the ECRHS I and II protocol (123). Measurement of IgE antibodies against birch was performed in all centers in the ECRHS I and in the Uppsala center in ECRHS II. In addition, IgE antibodies to a mix of food allergens (containing egg white, milk, cod fish, wheat, peanut, and soybean) were measured using the same technique and reagents in ECRHS I and II (Pharmacia CAP System, MultiCap fx5, Thermo Fisher Scientific, Uppsala, Sweden). Serum with a positive reaction to the food panel (fx5) was further analyzed for the single allergens in the panel. The results are presented in kU/L, and values of 0.35 kU/L and above were regarded as positive sensitization (131). A titer below the detection level (< 0.35 kU/L) was arbitrarily given the value 0.17 kU/L. All samples from ECRHS I from Reykjavík, Uppsala, Gothenburg and Umeå were analyzed in Uppsala, as previously described (39), while samples from the random sample of ECRHS II (depending on availability of residual samples) were analyzed within the frame of EuroPREVALL in Manchester, as described elsewhere (133).

In Paper IV, the presence of IgE antibodies was examined using both an allergen extract-based method (22) for birch, timothy, Dermatophagoides pteronyssinus, Cladosporium herbarum and cat (ImmunoCAP system: Immunodiagnostics, Thermo Fisher Scientific, Uppsala, Sweden) and chip technology (ImmunoCAP ISAC 103; Thermo Fischer Scientific) (129). Dermatophagoides pteronyssinus and Cladosporium herbarum are not presented in the paper due to the small number of sensitized subjects to the respective component allergens (3.2 % and 0.2 %, as previously reported (134)). The sub-analyses focusing on the groups with negative sensitization to allergen components but positive to extract were done only for timothy and cat, as this group was very small for birch. A subject was defined as IgE-sensitized to an allergen extract in case of IgE values ≥ 0.35 kU/L for this allergen (131). Subjects with IgE antibody levels < 0.35 kU/L (the lower limit of detection) were given an arbitrary value of 0.17 kU/L. Regarding component sensitization, subjects were considered non-IgE-sensitized if the measurement was lower than 0.3 ISU. The IgE antibody levels were added together (Sum IgE) (22) in order to get an assessment of the overall levels of IgE sensitization to components of timothy, birch and cat, respectively. 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 of each respective allergen.
Blood eosinophil count

Blood eosinophil count (B-Eos) was measured with a clinical routine method (Cell-Dyn Sapphire, Abbott, Illinois, USA).

Measurements of exhaled NO

In Papers I, III and IV, FeNO measurements were performed in accordance with the American Thoracic Society/European Respiratory Society recommendations (135) using an exhalation flow rate of 50 ml/s, with the exception that no initial vital capacity maneuver was performed, as a deep breath with slow inhalation was found to be sufficient (136). The system used for NO measurements was a computer-based single-breath NO system from Nitrograf (Hässelby, Sweden) with a chemiluminescence analyzer (Sievers NOA 280; Sievers, Boulder, Colorado, USA) in Papers I, III and IV, and NIOX® Flex; Aerocrine AB, Solna, Sweden in Paper II. Measurements were performed in duplicate and if the difference between individual measurements exceeded 10 %, additional measurements were done.

Smoking history

Smoking history was assessed by questionnaire. In ECRHS subjects who have smoked at least one pack-year and were still smoking were defined as current smokers. In MIDAS study current smokers were defined as individuals reporting current smoking of at least one cigarette/day during the last 6 months prior to the study.

Lung function

In Papers I, III and IV, forced expiratory volume in one second (FEV₁) was measured using a dry rolling-seal spirometer (Model 2130; Sensor Medics, Anaheim, California, USA). Up to five technically acceptable maneuvers were measured. In Paper II, flow-volume curves were performed using a Master Scope spirometer (Erich Jaeger, Wurzburg, Germany). Measurements were performed in accordance with the ATS/ERS recommendations (137).
Methacholine challenge test

Details of the methacholine challenge test have been described previously (12). In Papers I and IV, the level of airway hyperresponsiveness was expressed as a dose-response slope (55), where a lower value indicates a higher degree of airway hyperresponsiveness.

In Paper II, bronchial provocation tests were performed with Aerosol Provocation System (Viasys Healthcare GmbH) using a simplified protocol (138). Two minutes after each inhalation, FEV₁ was measured and PD20 FEV₁ (the cumulative dose of methacholine causing a 20 % reduction in FEV₁) was calculated by logarithmic interpolations using an integrated program.
Statistical methods

Statistical analyses were performed using STATA 11.0 software (Stata Corp., 2001, Texas, USA) in Paper I and STATA 12.1 software (Stata Corp., College Station, Texas, USA) in Papers II, III and IV.

In Paper I, logistic regression was used to analyze the correlation between the type of sensitization and asthma. Linear regression was used when analyzing correlation between the type of sensitization and exhaled NO and correlation between sensitization and airway hyperresponsiveness. These correlations were further tested in models that included age, sex, body mass index and smoking history.

In Paper II, we used percentage differences of FeNO, PD_{20} and B-Eos as a measure of effect. Percentage differences of FeNO, PD_{20} and B-Eos were obtained from linear regression models, where the log-transformed value was the outcome variable. The regression coefficient for the predictor variable of interest (e.g., food sensitization) was back-transformed, as described in previous studies (21), in order to obtain fold differences of FeNO, PD_{20} and B-Eos (e.g., food sensitized vs. non-food-sensitized), and these were in turn converted to percentage differences by multiplying the obtained fold differences by 100 and subtracting 100 %. We estimated percentage differences of FeNO, PD_{20} and B-Eos for every 1 % increase of grade of IgE sensitization by back-transforming the regression coefficient from the model where both outcome and predictor were log-transformed (139).

In Paper III, multiple logistic regression models were used when examining predictors for new-onset food hypersensitivity, and persistent food sensitization. These models always included age, sex, body mass index, rhinitis, current asthma, total IgE, IgE against food allergens, sample and IgE against aeroallergens.

In Paper IV, linear regression was performed to assess correlation between log-transformed titers 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 percent agreement (PPA), i.e., the percentage of subjects positive to allergen components (> 0.3 ISU) out of all subjects who were positive using the extract-based method at either a cut-off of > 0.35 kUA/L or an arbitrarily chosen (114, 115) higher cut-off of > 1 kUA/L, and of the negative percent agreement (NPA), i.e., the percentage of subjects who were negative to allergen components (< 0.3 ISU)
out of all subjects who were negative to the extract. A p–value of < 0.05 was considered statistically significant.
Ethics

All subjects gave their written permission for the utilization of personal data for the purpose of these studies. Studies within Papers I, II, III and IV were approved by the Ethics Committee at the Medical Faculty at Uppsala University. The study in Paper III was also approved by the Ethics Committee at University of Iceland.
Results

Paper I

Prevalence of IgE sensitization to various allergens

Of the 103 allergen components on the ISAC chip, 78 were detected in at least one of the tested individuals. IgE antibodies against at least one component were detected in 141 (38.0 %) of the non-asthmatic and 70 (72.9 %) of the asthmatic subjects (p < 0.0001). The asthmatic subjects had a significantly higher prevalence of IgE antibodies against foods of plant origin, grass, tree and weed pollen, furry animals, mold, latex and other allergens (p < 0.05).

Independent association of IgE sensitization to various groups of allergens with asthma, FeNO and airway hyperresponsiveness

Multiple variable analyses were performed with the allergen components merged into four groups, as presented in Table 3. Asthma was independently related to having IgE against pollen and perennial allergens, increased FeNO was independently related to having IgE against food allergens and perennial allergens, while increased airway responsiveness was independently associated only with IgE against perennial allergens. These results were consistent in a similar model where sum ISU titers for allergens within the respective categories were calculated for each allergy category and inserted in the multiple linear regression models as predictors.
Table 3. Independent association between having specific IgE against groups of allergen components and asthma, exhaled NO and airway hyperresponsiveness, expressed as dose-response slope to methacholine challenge, where a negative value indicates greater responsiveness.

<table>
<thead>
<tr>
<th>Category</th>
<th>Asthma (OR (95 % CI)#</th>
<th>FeNO (% difference (95 % CI))#</th>
<th>Slope (Beta (95 % CI))#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food (foods of plant and animal origin)</td>
<td>1.20 (0.69-2.32) (n = 118) §</td>
<td>20.2 (2.3,44.5) (n = 70)</td>
<td>-0.32 (-0.86, 0.23) (n = 81)</td>
</tr>
<tr>
<td>Pollen (grass, tree and weed)</td>
<td>2.21 (1.20-4.07) (n = 143)</td>
<td>12.2 (-4.5, 34.9) (n = 82)</td>
<td>-0.20 (-0.75, 0.34) (n = 108)</td>
</tr>
<tr>
<td>Perennial (animal, mite, mold)</td>
<td>5.59 (3.10-10.1) (n = 87)</td>
<td>47.9 (25.9, 77.8) (n = 57)</td>
<td>-1.43 (-2.03, -0.83) (n = 65)</td>
</tr>
<tr>
<td>Other (cockroach, latex, other)</td>
<td>2.02 (0.76-5.35) (n = 25)</td>
<td>9.6 (-18.7,47.9) (n = 14)</td>
<td>-0.80 (-1.76, 0.17) (n = 19)</td>
</tr>
</tbody>
</table>

#Adjusted for the variables in the table and age, sex, body mass index and smoking history.
§ Number of subjects sensitive to respective allergen among subjects with recorded asthma status (n = 467), FeNO (n = 288) or slope (n = 362).
*p < 0.05

The combined effect of IgE sensitization to different groups of allergens was analyzed by combining sensitization to the different allergen groups (Figure 3). A combination that included IgE antibodies against perennial allergens was independently associated with asthma. Sensitization to food allergens was related to asthma and increased FeNO in subjects who were also sensitized to pollen and/or perennial allergens. The highest association to asthma, FeNO and airway hyperresponsiveness was found in subjects who had IgE antibodies against components from all three of these groups (Table 4).
Figure 3. Asthma prevalence (percentage, left panel), FeNO levels (geometrical mean (95 % CI) on a log-scale, middle panel) and airway hyperresponsiveness (mean +/- SEM, right panel) in relation to sensitization to food, pollen, and perennial allergen components. * p < 0.05 compared with the group without any allergic sensitization

Table 4. Asthma, exhaled NO and airway hyperresponsiveness expressed as dose-response slope to methacholine challenge, where a negative value indicates greater responsiveness in relation to sensitization (mean (95 % CI)).

<table>
<thead>
<tr>
<th>Food §</th>
<th>Pollen $</th>
<th>Perennial £</th>
<th>N</th>
<th>Asthma (OR (95 % CI))#</th>
<th>FeNO (% difference (95 % CI))#</th>
<th>Slope (Beta (95 % CI))#</th>
</tr>
</thead>
<tbody>
<tr>
<td>No No No</td>
<td>0.49 (0.16-2.02)</td>
<td>2.3 (-18.7, 31.8)</td>
<td>-0.01 (-0.82, 0.79)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Yes No</td>
<td>1.67 (0.68-4.10)</td>
<td>2.3 (-20.6, 28.8)</td>
<td>-0.23 (-0.91, 0.46)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No No Yes</td>
<td>8.18 (3.00-22.3)*</td>
<td>62.2 (23.0,118)*</td>
<td>-1.78 (-2.81, -0.76)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes Yes No</td>
<td>4.43 (1.95-10.1)*</td>
<td>73.8 (34.9,123)*</td>
<td>-0.82 (-1.68, 0.04)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Yes Yes</td>
<td>8.69 (3.32-22.8)*</td>
<td>66.0 (23.0,123)*</td>
<td>-1.18 (-2.13, -0.23)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes No Yes</td>
<td>2.22 (0.24-20.8)</td>
<td>118 (17.5, 307)*</td>
<td>-0.35 (-2.60, 1.90)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes Yes Yes</td>
<td>18.3 (7.83-42.5)*</td>
<td>90.5 (47.9, 139)*</td>
<td>-2.41 (-3.23, -1.60)*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

# adjusted for age, sex, body mass index and smoking history, *p < 0.05. § both plant and animal origin, $ grass, tree and weed, £ animal, mite, mold.

Paper II

Prevalence of IgE sensitization to various allergens

The prevalence of sensitization to all allergens was higher among asthmatics than among controls (p < 0.05). Sensitizations to cat, dog or timothy were the most common among asthmatics, with prevalence figures between 50 and 60 %, whereas hazelnut, birch and timothy, with prevalence figures of up to 20 %, were the most common sensitizations among controls.
Association between IgE sensitization and FeNO, airway hyperresponsiveness and B-Eos in asthmatics

FeNO, airway responsiveness and B-Eos were higher in sensitized asthmatics compared with non-sensitized asthmatics for all groups of allergens (all p-values < 0.05). FeNO was independently associated with the presence of IgE (≥ 0.35 kU/L) against mold, furry animals and food, after adjustment for type of sensitization, age, sex, height, smoking history and medication. Similarly, airway hyperresponsiveness was independently associated with having IgE against mold and furry animals, whereas B-Eos was independently associated with the presence of IgE against mold, furry animals and food (Figure 4). Furthermore, FeNO was independently associated with the degree (sum IgE) of furry animal and food IgE sensitization; airway hyperresponsiveness was independently associated with the degree of mold and furry animal IgE sensitization; and B-Eos was independently associated with the degree of mold, furry animal and food IgE sensitization.

Figure 4. Independent association (expressed as % difference (95% CI))* in FeNO, airway hyperresponsiveness (PD20) (expressed as dose-response slope to methacholine challenge, where a lower value indicates greater responsiveness) and B-Eos between subjects sensitized and non-sensitized to specific allergen groups (significant difference (p < 0.05) means that the line of error bar does not cross the zero-percentage difference line).

*Adjusted for the variables in the figure and age, sex, height, smoking history, use of inhalant corticosteroids and use of antileukotrienes.
§ No. of subjects sensitive to the respective allergens among asthmatics with FeNO (n = 402), PD20 (n = 352) or B-Eos(n = 402).

Paper III

Natural history of food hypersensitivity symptoms

Around 21 % of the interviewed subjects reported food hypersensitivity symptoms in both ECRHS I and II (Table 5). The most common foods relat-
ed to these symptoms were fruits, followed by nuts and then vegetables. The prevalence of food hypersensitivity in relation to ingestion of fish (including seafood and shellfish) increased significantly from ECRHS I to II and this became the fourth most common cause of food hypersensitivity, alongside vegetables. Food hypersensitivity symptoms due to dairy products, wheat products (gluten, cereal, and wheat) or spices (herbs, chili, and garlic) also increased significantly from ECRHS I to II (Table 5).

Table 5. Prevalence (n (%)) of type of food reported as cause of food hypersensitivity (n = 2,358).

<table>
<thead>
<tr>
<th></th>
<th>ECRHS I 1991-92</th>
<th>ECRHS II 2000-01</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any food</td>
<td>498 (21.1)</td>
<td>526 (22.3)</td>
<td>0.58</td>
</tr>
<tr>
<td>Fruits</td>
<td>209 (8.9)</td>
<td>225 (9.5)</td>
<td>0.31</td>
</tr>
<tr>
<td>Nuts</td>
<td>163 (5.3)</td>
<td>147 (6.2)</td>
<td>0.6</td>
</tr>
<tr>
<td>Vegetables</td>
<td>102 (3.4)</td>
<td>106 (4.5)</td>
<td>0.13</td>
</tr>
<tr>
<td>Fish, seafood, shellfish</td>
<td>75 (2.5)</td>
<td>106 (4.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Chocolate</td>
<td>38 (1.3)</td>
<td>23 (1)</td>
<td>0.31</td>
</tr>
<tr>
<td>Egg</td>
<td>33 (1.1)</td>
<td>20 (0.9)</td>
<td>0.34</td>
</tr>
<tr>
<td>Milk and dairy products</td>
<td>31 (1)</td>
<td>60 (2.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Meat</td>
<td>11 (0.4)</td>
<td>19 (0.8)</td>
<td>0.13</td>
</tr>
<tr>
<td>Gluten, cereal, wheat products</td>
<td>8 (0.3)</td>
<td>14 (0.6)</td>
<td>0.049</td>
</tr>
<tr>
<td>Herbs, chili, garlic</td>
<td>9 (0.3)</td>
<td>20 (0.9)</td>
<td>0.004</td>
</tr>
</tbody>
</table>
Prevalence of IgE sensitization to at least one food allergen decreased by 56 % during the study (Figure 5A). Peanut IgE sensitization had the highest prevalence among the food allergens; it decreased by 67 %. The prevalence of soy IgE sensitization decreased significantly between ECRHS I and II, whereas similar prevalence figures for IgE sensitization to wheat, milk, egg and fish were found at both time points (Figure 5B). No significant change of prevalence of cat, birch and mite IgE sensitization was found, whereas prevalence of timothy grass IgE sensitization decreased by 15 % (prevalence decrease from 17 % to 15 %) (Figure 5A).

**Figure 5.** Prevalence of sensitization to food (n = 807) and aeroallergens (n = 804) (panel A) and prevalence of IgE sensitization to individual food allergens (panel B) in ECRHS I and II. Birch pollen was measured only in Uppsala (n = 138).
Incidence of food hypersensitivity

The incidence of food hypersensitivity was 11.7 % during this 9-year study. The incidence in the symptomatic sample was higher than in the random sample (20.6 % vs. 9.9 %, p < 0.001). Female sex OR: 1.8 (1.3, 2.6), rhinitis OR: 1.8 (1.2, 2.6), eczema OR: 1.8 (1.3, 2.5), and presence of IgE sensitization to aeroallergens OR: 1.9 (1.3, 2.8) were associated with new-onset food hypersensitivity. Odds ratios were adjusted for the variables in the model and age, body mass index, current smoking, sample and total IgE.

Paper IV

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 (Figure 6). A total of 48 % of subjects who were sensitized to cat extract were not sensitized to any investigated cat allergen component. 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, versus 5.6 (4.3, 7.3) kU/L in subjects IgE-sensitized to both cat extract and components. 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. This high percentage of individuals not sensitized to cat components decreased to 23 % for cut-off > 2 kU/L IgE against cat extract and to 16 % for cut-off > 3 kU/L IgE against cat extract. For timothy, 21 % of the subjects who were sensitized to timothy extract were not sensitized to timothy components. The highest agreement was seen for birch, for which only 14 % of the extract-sensitized subjects were not sensitized to birch components. The 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 the 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 (Figure 6).
Figure 6. Contingency tables and scatter plots of IgE levels against 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.
**IgE sensitization patterns for cat and timothy in relation to new-onset rhinitis and asthma**

In multiple logistic regression models, new-onset rhinitis was associated with sensitization to cat components but not with sensitization to the extract (Figure 7A). This association remained significant when analyzing the additive value of sensitization to cat components in a model containing age, sex, BMI, smoking and asthma at baseline (Pseudo-R2 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 to both cat extract and cat components (Figure 7B). Similarly, there was a consistent additive explanatory value of IgE sensitization to cat components for new-onset asthma (Pseudo-R2 increased from 7 % to 22 %, \( p = 0.005 \)), but this was not found for the extract (\( p = 0.15 \)). 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.

**Figure 7.** Odds ratios for new-onset rhinitis\# and new-onset asthma* for subjects sensitized to cat allergens (Panels A & B). \#Adjusted for age, BMI, sex, smoking, asthma at baseline, *Adjusted for age, BMI, sex, smoking, rhinitis at baseline.
Relative importance of different cat and timothy components for airway inflammation and responsiveness and new-onset respiratory disease

An independent association was found between sensitization to Fel d 1 or Fel d 2 and FeNO (p = 0.002 and p < 0.001) and airway hyperresponsiveness (p = 0.001 and p = 0.02) when analyzing independent effects of IgE sensitization to cat components in the same model (Table 6). Regarding new-onset respiratory disease, only a trend towards an association between IgE sensitization to Fel d 1 and new-onset rhinitis (p = 0.083) was found (Table 6).

Regarding timothy components, only IgE sensitization to Phl p1 was independently associated with higher present increase in FeNO, [7.2 % (0.7, 14.9), p = 0.03] and airway hyperresponsiveness [regression coefficient: -1.1 (-1.9,-0.3), p = 0.009]. Furthermore, IgE sensitization to Phl p1 was associated with new-onset rhinitis [OR: 8.2 (1.2, 55.7), p = 0.03].

Table 6. Independent association between FeNO, slope (where lower values indicate more airway hyperresponsiveness) new-onset rhinitis# and new-onset asthma* and cat components in the same model.

<table>
<thead>
<tr>
<th>Components</th>
<th>rFel d 1 (% difference (95 % CI))</th>
<th>nFel d 2 (% difference (95 % CI))</th>
<th>rFel d 4 (% difference (95 % CI))</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeNO§</td>
<td>14.9 (7.2, 23.1)</td>
<td>23.1 (7.2, 41.4)</td>
<td>1.4 (-6.7, 7.2)</td>
</tr>
<tr>
<td>Beta (95 % CI)</td>
<td>Beta (95 % CI)</td>
<td>Beta (95 % CI)</td>
<td></td>
</tr>
<tr>
<td>Slope§</td>
<td>-1.5 (-2.4, -0.7)</td>
<td>-1.5 (-2.8, -0.2)</td>
<td>-0.5 (-1.5, 0.6)</td>
</tr>
<tr>
<td>OR (95 % CI)</td>
<td>OR (95 % CI)</td>
<td>OR (95 % CI)</td>
<td></td>
</tr>
<tr>
<td>New-onset rhinitis #</td>
<td>7.0 (0.8, 63.2)</td>
<td>Omitted</td>
<td>1.9 (0.1, 53.2)</td>
</tr>
<tr>
<td>New-onset asthma*</td>
<td>3.4 (0.3, 25.3)</td>
<td>7.6 (0.5, 110)</td>
<td>7.8 (0.6, 98.1)</td>
</tr>
</tbody>
</table>

#Adjusted for age, BMI, sex, smoking, asthma at baseline.
*Adjusted for age, BMI, sex, smoking, rhinitis at baseline.
§ Adjusted for age, BMI, sex, smoking.
Discussion

Asthma, degree of inflammation and airway hyperresponsiveness in relation to pattern of IgE sensitization in a general population and in a cohort of asthmatics

Having IgE antibodies against perennial aeroallergens was the type of sensitization that had the strongest relation to asthma, exhaled NO and airway hyperresponsiveness in a population-based study (Paper I). This result is in line with other analyses from the ECRHS cohort (22, 54, 55) and other studies (53, 58, 140). Sensitization to pollen was also an independent risk factor for asthma but not for elevated FeNO or increased airway responsiveness. The latter could be due to the fact that most subjects (80 %) in this population were examined outside the pollen season (141). Other studies have found that sensitization to pollen is less related to asthma and airway hyperresponsiveness compared with sensitization to perennial allergens (142). An increasing prevalence of asthma and higher exhaled NO levels and airway hyperresponsiveness could be found with increasing numbers of sensitizations, when grouped into perennial, pollen and food allergen sensitizations. This is in line with previous studies where multiple sensitization related to higher risk of asthma (8) and bronchial responsiveness (36).

In Paper II, we observed an association between IgE sensitization to aeroallergens and local and systemic inflammation in asthmatics. The markers of local inflammation, FeNO and airway responsiveness were independently associated with the presence of IgE antibodies against mold and furry animals. FeNO has previously been reported to be increased in asthmatic children sensitized to furry animals (63) and is also associated with the degree of IgE sensitization to furry animals in asthmatic children (63) and adults (23). Mold sensitization was related to high FeNO levels in a population-based study (22). The levels of FeNO were significantly higher in patients with asthma who were both sensitized and exposed to a relevant aeroallergen (mite, cat or dog) than in those who were sensitized but not exposed (143). However, in the present study, mite sensitization was not related to increased FeNO, which probably reflects the low exposure to mite allergens in Sweden (144).
In Paper I, we found an independent association of perennial (furry animals, mite and mold) IgE sensitization with airway hyperresponsiveness (134). Mold sensitization was the type of sensitization associated with the largest increase in airway hyperresponsiveness and previous studies have shown that mold sensitization is associated with more severe asthma in young adults (145) and more frequent admission to intensive care (146).

IgE sensitization against food allergens and exhaled NO and blood eosinophil levels

In Paper I, sensitization to food allergen components was independently associated with increased FeNO. In subjects dually sensitized to pollen and food allergens, an increased risk of asthma and elevated FeNO was seen, whereas no such association was found in subjects who were only sensitized to pollen. Previous studies have shown a significant association between food allergy and asthma, which was even stronger in children with multiple food allergies or severe food allergy. These findings suggest that food allergy could be an under-recognized risk factor for problematic asthma (85).

In Paper II, we could confirm the finding from the population-based study (Paper I) that food IgE sensitization is associated with increased airway inflammation. Although we have no data on food allergen exposure in Paper II, it is reasonable to assume that only a minority of the subjects with IgE antibodies towards a food allergen have symptoms of food allergy (64) and therefore avoid the food in question.

The marker of systemic eosinophil inflammation used in the present study, i.e., the number of blood eosinophils, was independently associated with the presence of IgE sensitization to mold, furry animals and food, and was also independently associated with the degree of mold, furry animal and food IgE sensitization. Development of eosinophilia is common in asthmatics with mold sensitization (147). Current exposure to furry animals also associates with blood eosinophilia (148), and multisensitization to furry animal-derived components associates with increased numbers of blood eosinophils in patients with severe asthma (149). The relation between food allergen sensitization and eosinophilia has not been studied previously in asthmatics, although such a relation has been reported in a population-based study of adults (39) and in children with food allergy (38) or atopic dermatitis (150). The systemic eosinophilic inflammatory component is of importance for asthma, as the number of eosinophils in peripheral blood and in bronchial lavage from subjects with asthma is associated with disease severity (48). We have recently reported that both blood eosinophil counts and FeNO are independently associated with wheeze and asthma events in a large American population survey (49).
Mechanistically, exposure of the gastrointestinal tract to food allergens may cause subclinical inflammation in the gastrointestinal tract with activation of Th-2 lymphocytes and production of Th-2 cytokines, such as IL-5, IL-4 and IL-13. These cytokines can drive inflammation in the primed tissue of the airway mucosa in subjects sensitized to both aeroallergens and food allergens through IL-4/IL-13 and upregulation of iNOS and blood eosinophilia via the IL-5 inflammatory pathway (151) (Figure 8). Additionally, some of the food allergens may be inhaled and exposure to aerosolized food has been reported to result in asthma symptoms in asthmatic children with food allergies (152).

![Figure 8. Proposed mechanism.](image)

**Figure 8.** Proposed mechanism.

Methodological issues and clinical utility of multiplex, component-based vs. limited panel of extract-based IgE testing

About 70 % of asthmatic subjects and 40 % of non-asthmatic subjects were IgE-sensitized. Previous analyses of IgE sensitization in the ECRHS II population have only included four individual allergens: mite, cat, timothy and *Cladosporium* (22). By using the microarray technique, we identified IgE antibodies against almost 80 components in this population. This increased the prevalence of IgE sensitization from 28 % to 38 % in the non-asthmatic group, but had a relatively limited effect in the asthmatic group, where the prevalence of IgE sensitization only increased from 70 % to 73 %. A possi-
ble explanation is that the four allergens tested previously are the most common allergens causing mono-sensitization. Therefore, co-sensitization to other allergen components tested in the microarray does not increase the prevalence of atopy to a large extent. This is supported by a previous study from ECRHS (12). The results in the asthma group suggest that there is a proportion of 20-30 % of asthmatics who are not IgE-sensitized even when extended IgE characterization is performed. Additional information in terms of the number of allergen components a subject is sensitized to is obtained using the microarray, and this has been reported to be related to the risk of developing allergic disease (153).

The high overall correlation between IgE sensitization to allergen extract and the corresponding components in Paper IV is in agreement with previous studies on measurements of IgE towards single allergen components, which have shown strong agreement with extracts of birch (117), timothy (118, 119), and cat (120, 122). Further, high concordance has been shown between the use of timothy extract and allergen components measured with multiplex technique (114, 115). However, in our study, large variations in the positive percent agreement between the methods were noted, ranging between 52 % and 85 %, with the lowest PPA for cat. The PPAs for timothy and cat were lower than those presented by Bonini et al (114) and Melioli et al (115). Given that these two studies and our study use the same version of the ISAC microarray (with 103 allergen spots), 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 the present study, 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 generally had 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 to levels higher than 1, 2 or 3 kU/L.

Cat and timothy components’ predictive value for the risk of higher airway inflammation and hyperresponsiveness and development of new-onset respiratory disease

Assessment with cat components had a higher predictive value for the risk of developing asthma and rhinitis than analyses with 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 less
clinical importance. 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 airway hyperresponsiveness, while cat components Fel d 1 and Fel d 2 were associated with higher airway hyperresponsiveness and FeNO. A previous study has also shown the association of Phl p 1 with new-onset rhinitis (154), but no other study examined the association between timothy or cat components and new-onset rhinitis, FeNO or airway hyperresponsiveness. Another explanation may be that analysis of allergen components by multiplex technique is less sensitive (115), 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 added 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 (60, 155), and IgE to cat was related to new-onset asthma (57). To our knowledge, only one study has examined the prognostic value of IgE sensitization to allergen components. This study was performed in children and found a relation between timothy components and new-onset rhinitis (154).

Change in the prevalence of IgE sensitization to food allergens

A novel finding of Paper III was the marked reduction of prevalence of IgE sensitization to food allergens over time in adults. The most prominent decrease was noticed for peanut and soy, foods which sensitized subjects can probably avoid more easily than wheat, milk and fish. The natural history of prevalence of IgE sensitization to food allergens has previously been studied only in children (72). For aeroallergens, the natural history of prevalence of IgE sensitization has been examined in longitudinal studies in adults (156-162), but the results are contradictory and show the prevalence decreasing (159, 162), remaining stable (156, 163) or increasing (157, 158, 160, 161) over time. The decrease in prevalence of IgE sensitization to food allergens does not appear to be related to the initial age of the subjects, which tends to preclude the possibility that the effect is related to a specific birth cohort. Reductions of similar size could be observed in both younger and older sub-
jects. This could indicate that the decreasing prevalence is related to aging. Previous longitudinal studies (156, 158, 160, 161) on the prevalence of IgE sensitization to aeroallergens in relation to age have reported mutually inconsistent results.

Reported food hypersensitivity symptoms after food intake

To our knowledge, no previous studies have analyzed longitudinal changes of self-reported perceived food hypersensitivity in adults. The prevalence of food hypersensitivity reported was in line with reports from previous cross-sectional studies (67-69, 164). Our results, showing an unchanged prevalence of food hypersensitivity over time, are contrary to what is known about the natural history of food hypersensitivity in children, where major remission of symptoms has been reported (165-167).
Study limitations

In Paper I, enrichment of the randomly selected study group with a symptomatic sample (22) increased the group of asthmatics and allowed the analysis of relationship to asthma, but may have made the results less generalizable to general populations. The measurements of exhaled NO were not available in all subjects and therefore some of the analyses with regard to airway inflammation might have been underpowered.

In Paper II, the lack of data on exposure to allergens, especially food allergens, was a study limitation. The use of component-resolved diagnosis could have given more information regarding cross-reactivities. Simply summing up IgE titers against different allergens does not take into account primary sensitization versus cross-reactivities. Furthermore, component-resolved diagnosis would have allowed us to analyze if some allergen components for the same allergen were more important than others in relation to local and systemic inflammation.

A weakness of Paper III is that we were not able to assess the persistence/remission of food hypersensitivity as the questions referred to ever having had food hypersensitivity. A limitation is the fairly limited number of participants for whom we had data from repeated measurements of IgE against food allergens. Another limitation is that several of the most common food allergens in adulthood, such as hazelnut, shrimp, peach and apple (133), were not analyzed at both time points.

A weakness of Paper IV 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.
Future research

Molecular-based analysis of IgE sensitization can enhance the determination of IgE through extract-based tests. Large-scale multicenter studies will help identify which groups of patients are appropriate for this type of IgE testing. Furthermore, the value of specific allergen components to longitudinally predict new-onset/persistent respiratory symptoms remains to be examined further and in greater detail among asthmatics and general populations. Some allergen components from the same allergenic source can be more clinically important than others in relation to local and systemic inflammation. Such components must be spotted in order to identify atopic subjects who are going to develop allergic disease, use more healthcare and require more intense therapy in the future.
Conclusions

1. The type and degree of IgE sensitization to aeroallergens were important predictors of asthma, airway inflammation and reactivity.

2. Microarray chips marginally increased the information about prevalence (yes/no) of IgE sensitization compared with a standard panel of a limited number of allergens.

3. The known relationship of IgE sensitization against perennial allergens with airway inflammation, airway hyperresponsiveness and blood eosinophilic count was confirmed in children and young adults with asthma (Figure 9A).

4. IgE sensitization to food allergens is associated with increased local inflammation in a population-based material and to increased local and systemic inflammation in an asthmatic population (Figure 9B).

5. The prevalence of IgE sensitization to food allergens in adults decreased over a 9-year period, while the prevalence of food hypersensitivity remained unchanged.

6. Measurement of IgE sensitization to cat allergen components appeared 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.
Figure 9. Graphical summary of the main findings. We confirmed previous findings that IgE sensitization against perennial allergens relates to increased airway inflammation, airway hyperresponsiveness and blood eosinophilic count (Panel A). Our data support the new finding that IgE sensitization against food allergens also relates to increased airway inflammation and blood eosinophilic count (Panel B).
Clinical implications

Studies of the association between IgE sensitization profiles and local and systemic inflammation may enable personalized, biomarker-based treatment of patients with asthma in the future. Different allergy profiles can associate to differing extents with the various features of respiratory disease, suggesting that they also have differing therapeutic needs. Identifying these needs can be the first step towards individually tailored treatments.

Multiplex component analysis is of clinical use in identifying true food allergies. Its epidemiological use remains to be established, and in Paper I we have used only the information regarding perennial, pollen and food sensitization, not the full information regarding the degree of sensitization to all allergen components. By taking advantage of information about primary sensitization and cross-reactivity as well, it would be possible to get a detailed allergic profile of a population and the relation of these sensitizations with ongoing respiratory disease, airway inflammation and responsiveness.

In Paper IV, we identified allergen components that were associated with new-onset respiratory symptoms and airway inflammation and responsiveness. Such information can enable physicians to detect patients who would be affected more severely and require more healthcare in the future. That in turn would help choosing appropriate candidates for immunotherapy and other therapies, improving their efficacy.
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