Specific IgE and IgG measured by the MeDALL allergen-chip depend on allergen and route of exposure: The EGEA study

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Abstract

Background: The nature of allergens, route and dose of exposure may affect the natural development of IgE and IgG responses.

Aim: To investigate the natural IgE and IgG responses towards a large panel of respiratory and food allergens in subjects exposed to different respiratory allergen loads.

Methods: A cross-sectional analysis was conducted in 340 adults of the EGEA cohort (Epidemiological study of the Genetics and Environment of Asthma, bronchial hyperresponsiveness and atopy) (170 with and 170 without asthma). IgE and IgG to 47 inhalant and food allergen components were analyzed in sera using the MeDALL micro-array technology and compared between 5 French regions according to the route of allergen exposure (inhaled vs. food allergens).

Results: Overall 48.8% of the population had allergen-specific IgE≥0.3 ISU to at least one of the 47 allergens with no significant differences across the regions. For ubiquitous respiratory allergens (i.e., grass-, olive/ash pollen, house dust mites), specific IgE did not show marked differences between regions and specific IgG (≥0.5 ISU) was present in most subjects everywhere. For regionally occurring pollen allergens (ragweed, birch, cypress), IgE sensitization was significantly associated with regional pollen exposure. For airborne allergens cross-reacting with food allergens, frequent IgG recognition was observed even in regions with low allergen prevalence (Bet v 1) or for allergens less frequently recognized by IgE (profilins).

Conclusions: The variability of allergen specific IgE and IgG frequencies depends on exposure, route of exposure and overall immunogenicity of the allergen. Allergen contact by oral route might preferentially induce IgG responses.

Key messages

IgE and IgG towards respiratory allergens are usually associated with inhalant allergen exposures but IgG is frequent in subjects exposed to cross-reacting food allergens. Oral exposure preferentially induces IgG responses.

Capsule summary

Inhalant and oral routes of exposure induce different patterns of sensitization. Oral route may preferentially induce IgG responses whereas respiratory route is important for IgE sensitization. A geographical pattern of IgE response to pollen exposure exists in France, depending on regional pollen exposure.

Key words

IgE, IgG, allergen components, respiratory allergens, food allergens, EGEA, cohort, epidemiology, MeDALL, microarray

Abbreviations

Bet v: Betula verrucosa

Cyn d: Cynodon dactylon

EGEA: Epidemiological study of the Genetics and Environment of Asthma, bronchial hyperresponsiveness and atopy

Fel d: Felis domesticus

FP7: Framework Programme 7 (European Union)

IgE: Immunoglobulin E

IgG: Immunoglobulin G

Mal d: Malus domestica

MeDALL: Mechanisms of the development of allergy

Phl p: Phleum pratense

PR-10: pathogenesis-related protein family PR-10

Introduction

Allergic patients are characterized by the production of specific immunoglobulin E (IgE) antibodies against allergens [1] whereas allergen-specific IgG antibodies occur in both allergic and non-allergic individuals.[2] Allergen-specific IgG antibodies play a role in allergen-specific clinical tolerance, either occurring naturally or induced by allergen-specific immunotherapy.[3-7] For certain allergens IgE and IgG responses differ.[8] Allergen-specific IgG may be associated with exposure to the respective allergen.[9,10] However, population data regarding the role of IgG production to a broad panel of common food and inhalant allergens are not available.

The route and dose of exposure to allergens are highly relevant for IgE and IgG sensitization. Oral route may favour an IgG response whereas inhaled exposure may favour an IgE response.[8,11] Investigating the IgG response to several molecule groups like profilins (e.g., Bet v 2, Phl p 12), including both inhalant and food allergens, or polcalcins, i.e., calcium-binding allergens (e.g., Bet v 4, Phl p 7), including only inhalant allergens, could provide additional insight into the role of the route of exposure for allergic sensitisation.

The microarray technology allows measurement of allergen-specific IgE and IgG antibody responses to many allergen components. In MeDALL, the ImmunoCAP ISAC chip technology was improved to increase its sensitivity and to incorporate new allergens.[12-16] The resulting MeDALL chip has been validated [15,16] and more than 6000 sera from 7 European birth cohorts (e.g., BAMSE and ECA)[17,18] have been tested so far.

The Epidemiological study of the Genetics and Environment of Asthma, bronchial hyperresponsivness and atopy - EGEA [19-21] is a cohort of adults and children with and without asthma recruited in five French cities (Paris, Lyon, Grenoble, Montpellier and Marseille). Pollen exposure varies widely among these different places. While grass pollen exposure is similar across France, exposure to pollens of birch, cypress and ragweed exhibits strong geographical variability.

As part of the MeDALL project,[15] the present study investigates the geographical variability of allergen-specific IgE and IgG towards a large variety of different respiratory and food allergens using the MeDALL micro-array technology in sera from the EGEA

155 cohort obtained from subjects recruited in different places in France. We addressed if
156 there are differences among the types of allergens, routes of allergen exposure and levels
157 of respiratory allergen exposure on allergen-specific IgE and IgG production.
158

Methods

Population and study setting

The EGEA cohort is composed of patients with asthma enrolled in chest clinics and their first-degree relatives, and a group of control subjects, all recruited in the early 1990s in 5 French cities. Protocol and characteristics have been previously published.[22,23] Briefly, 2047 participants were enrolled at baseline (EGEA1) including 388 children (<16 years) and adult patients with asthma from chest clinics, their 1244 first-degree relatives and 415 population-based control subjects. Subjects were recruited through self-completed questionnaires and an overall matching by months at exam, age (decade), sex and centre. About 11 years later, this population was invited for a follow-up (EGEA2) and 1601 participants (77.1% of the original cohort + 58 new family members) were subjected to a complete examination, which included serum samples.[21] EGEA collection is certified ISO 9001 and referenced in the Biobank network by the number BB-0033-00043.

The present analysis was conducted in 340 adult participants obtained at the follow-up (i.e., EGEA2) (170 with and 170 without asthma). Participants were randomly selected among all followed-up subjects with available serum and data on genetic polymorphisms (to facilitate further analyses), skin prick tests, and total IgE at baseline (EGEA1) and follow-up (EGEA2) visits and non-missing data for asthma status.

Written consent was obtained from all individuals. Ethical approval was obtained for both surveys (Cochin Royal Hospital, Paris for EGEA1 and Necker-Enfants Malades Hospital, Paris for EGEA2).

Asthma, allergic rhinitis phenotypes and Skin Prick Tests (SPT)

The definition of ever asthma was based on a positive answer to either ‘‘Have you ever had attacks of breathlessness at rest with wheezing?’’ or ‘‘Have you ever had asthma attacks?’’ or being recruited as an asthma case.[23] Allergic rhinitis ever was defined by a positive answer to “Have you ever had allergic rhinitis?” or “Have you ever had hay fever?”. Subjects with a positive answer to “Have you had a problem with sneezing or runny or blocked nose when you did not have a cold or flu in the last 12 months” have been defined as active allergic rhinitis.

SPT was performed for 11 allergens (cat, *Dermatophagoides pteronyssinus*, *Blattela*

germanica, olive, birch, *Parieteria judaica*, timothy grass, ragweed pollen, Aspergillus, *Cladosporium herbarum*, *Alternaria tenuis*). Positive SPT was defined by a mean wheal diameter ≥3mm.

Measurement of allergen-specific IgE and IgG with the MeDALL allergen-chip

IgE and IgG were determined in anonymized samples with the MeDALL-chip as described.[15,16] It comprises 176 allergen components including aero- and food allergen components. The measurement of allergen-specific IgE- and IgG reactivities was performed as described.[16,18,24].

The present study is focused on outdoor allergen sources whose distribution varied in the different geographical areas (birch pollen, cypress pollen, ragweed pollen, olive/ash pollen, timothy grass pollen), and on allergens from indoor allergen sources (house dust mites). We studied IgG to the PR-10 group of allergens with cross-reactive food allergens.[18] To further address the hypothesis that allergen contact mainly by the oral route induces IgG response, three common food allergens were also studied (milk, egg, fish) allowing to compare the frequency of IgE and IgG recognition across respiratory and food allergens. Overall, 47 representative allergen components were analysed in detail (Table 2).

Geographical exposure to allergens

The geographical distribution of the studied pollens across France in 2006 was obtained from the RNSA (Réseau National de Surveillance Aérobiologique), the French aerobiology network in charge of the analysis of biological particles in air samples (http://www.pollens.fr).[25,26] Data are presented as the daily mean of grains/m^3 across the year 2006, a year included in the EGEA2 data collection period (2003-2007).

Biases

The random selection of the EGEA2 participants to the present analysis was *a priori* unrelated to allergen exposure and allergic status, minimizing the risk for selection bias. In both subjects with and without asthma, subjects included in the analysis did not differ regarding allergic sensitization as defined by ≥1 positive reaction in SPT using 12 different extracts, level of total IgE and allergic rhinitis phenotype as compared to non-included participants (Table E1). Regarding exposure we did not observe marked differences

between included and non-included subjects, although included subjects with asthma were more often recruited in Lyon and Grenoble (Table E1).

Statistical methods

According to previous studies, IgE and IgG positivity to allergen was defined by a threshold of 0.3 ISU and 0.5 ISU, respectively[16-18]. Ordinal categorical variables were also studied both for IgE (<0.3 <1 <3 <10 <30 and ≥ 30 ISU) and IgG (<0.5 <1.5 <5 <15 <50 and ≥50 ISU) because of the non-Gaussian distribution. Chi^2 test was computed when applicable. Non-parametric Kruskal-Wallis tests were also applied to address whether the IgE and IgG levels (considered as continuous outcomes) differed among centres. Odds Ratios (OR) were estimated to assess the magnitude of the association between centres and allergen-specific IgE (or IgG) sensitization using multinomial logistic regression adjusted on age, sex, asthma and rhinitis status.

Results

Characteristics of study subjects

In this study, 340 sera from different centers (Paris: n=96; Lyon: n=75; Marseille: n=41; Montpellier: n=22; Grenoble: n=106) were available. The demographic characteristics of participants are presented in Table 1. The population included 47% men and the mean age was 43 years. Fifty-two percent of participants had allergic rhinitis ever and 37.6% had atopic dermatitis ever. Eighty-two participants reported to have received some form of allergen immunotherapy (AIT).

Most frequently recognized allergens by IgE

Overall, 48.8% of the population had allergen-specific IgE ≥0.3 to at least one of the allergen components selected, which is in strong agreement with the prevalence of atopy (defined by positive SPT to at least one of 11 aeroallergens) in this population (Cohen Kappa coefficient = 0.68). The agreement between SPT and allergen-specific IgE was strong for each allergen (table E2). Pollen allergens most frequently recognized by IgE were Phl p 1 (33.8%), Phl p 4 (22.4%), Ole e 1 (20.9%), Phl p 5 (18.2%), Cup a 1 (18.2%), Phl p 2 (15.3%), Phl p 6 (14.4%) and Bet v 1 (10%) (Table 2). The indoor allergens most frequently recognized by IgE were Der p 2 (27.4%), Der p 23 (27.4%), Der p 1 (24.4%), Fel d 1 (23.8%), Der p 5 (16.5%), Der p 4 (15.9%), Der p 7 (13.2%), Can f 5 (11.2%), Der p 21 (10%) (Table 2). Regarding food allergens, the family of PR-10 proteins (i.e., Mal d 1: apple; Cor a 1.0401: hazelnut; Ara h 8: peanut; Pru p 1: cherry; Gly m 4: soy) that cross-react with the major birch pollen allergen Bet v 1 were most frequently recognized by IgE (7.6 – 2.4% patients), followed by Gal d 1 (ovomucoid, egg) and Bos d 4 (lactalbumin, milk) with 0.6% and 0.3%. These patterns where consistent in the population who did not receive AIT, although the prevalence of IgE recognition was lower as compared to the whole study population (Table 2).

Similarities and discrepancies between IgE and IgG recognition

Distinct patterns of IgE and IgG recognition frequencies were found (Table 2). In general, respiratory allergens (Ole e 1, Phl p 1, Phl p 4, Der p 1, Der p 2, Der p 23, Fel d 1) frequently recognized by IgE antibodies (≥ 20% subjects) were frequently recognized by IgG as well (≥ 60% subjects). Likewise, some respiratory allergens less frequently

recognized by IgE antibodies (≤6% subjects), were also recognized at low frequency (i.e., <40%) by IgG (Phl p 7, Phl p 11, Bet v 4, Ole e 7, Amb a 1). On the other hand, other respiratory allergens also less frequently recognized by IgE antibodies were recognized in >80% by IgG (Phl p 12, Bet v 2, Ole e 9). Similar patterns were observed in the population without AIT.

Geographical variation of IgE and IgG recognition

Although the IgE sensitisation to at least one of the analysed allergens (Table 2) did not vary significantly across the geographical areas (from 39.0% to 54.7%, p=0.46), we observed considerable geographical differences for some specific allergens (Fig. 1 and Table 3).

For several major respiratory allergens the IgE and IgG frequencies in the different regions followed to a large extent the exposure levels with the corresponding allergen sources (e.g., ragweed exposure: Amb a 1; grass pollen exposure: Phl p 1 and exposure to cypress: Cup a 1) (Fig. 1 and table 3). For example, in agreement with similar levels of grass pollen exposure across France, IgE recognition frequencies for the major timothy grass pollen allergen, Phl p 1, were similar across the geographical areas (the adjusted-ORs for each center as compared to Paris varied between 0.68 to 1.29, p >0.50; Table 3) and IgG recognition of Phl p 1 was higher than 90% in each area. Inversely, IgE and IgG recognition frequencies for the cypress pollen allergen Cup a 1 showed marked geographical differences with adjusted OR of 7.4 (95%CI 2.1, 25.3) and 7.8 (95% CI 0.98-62.2) in Montpellier, the city showing the highest level of pollen exposures, as compared to Paris. Similar patterns were observed in subjects with and in subjects without asthma (Table E3), as well as in subjects without AIT (Table E4), although results should be interpreted cautiously because of the sample size .

However, for Bet v 1 the geographic pattern of IgE and IgG recognition differed. IgE recognition frequencies to Bet v 1 followed the exposure levels of birch pollen in the different regions whereas IgG recognition was high (>60%) in all regions. This was also observed for food allergens that showed immunological cross-reactivity with Bet v 1 (PR-10 proteins, e.g., Mal d 1 from apple, Cor a 1.0401 from hazelnut, Ara h 8 from peanut, Pru p 1 from cherry, Gly m 4 from soy) (Fig. 1).

293 Regarding ubiquitously occurring allergens such a highly cross-reactive plant allergens
294 (calcium-binding allergens: Phl p 7, Bet v 4; profilins: Phl p 12, Bet v 2), house dust mite
295 allergens (e.g., Der p 1, Der p 2, Der p 23, Der p 10, Der p 11, Der p 14) and food allergens
296 whose sensitization occurs primarily by oral route (e.g., Bos d 4, Gal d 1, Gad c 1), we
297 found little geographic variation of IgE and IgG recognition frequencies (Fig. 1 and 2).
298 Nevertheless, for some of these allergen components the IgG concentration varied across
299 the regions (as indicated by the non-parametric Kruskal-Wallis test estimated on
300 the continuous variables) and IgE reactivity against major indoor allergens from house
301 dust mite (i.e., Der p 2, Der p 23, Der p 1) tended to be lower in Lyon and Marseille (Fig.
302 2). In each region, Der p 10, Der p 11 and Der p 14, for which exposure also occurs via the
303 gut (Der p 10 cross-reacts with tropomyosin from shrimp) or the skin, were rarely
304 recognized by IgE but frequently by IgG (Table 2, Fig. 2).

305 Very few subjects showed IgE reactivity to allergens of milk (Bos d 4) and egg (Gal d 1),
306 and these allergens were recognized by IgG in about 90% of the subjects with no
307 differences across the geographical areas (Fig. 3).
308

Discussion

Microarrayed allergens are increasingly used to determine the profile of molecular IgE sensitization in populations.[27,28] However, to the best of our knowledge, the current study is the first systematic study comparing IgE and IgG responses to a large panel of respiratory and food allergens in a geographically defined population. It shows a large geographical variability in the distribution of IgE and IgG antibodies in France which could be summarized as following: 1) for ubiquitous allergens such as pollens of grass, olive/ash and to a lesser extent allergens from house dust mites, IgE antibodies were scattered around the different French regions (up to 34% of participants) and IgG was present in most subjects without any geographical difference (over 85%), 2) on the other hand, for regional pollen (ragweed, birch, cypress), IgE responses mirrored regional pollen exposure, but IgG response varied depending on allergen sources: high for pollen allergens cross-reacting with food allergens (Bet v 1 and other PR-10 allergen components) and glycosylated components (e.g., Cup a 1 and Phl p 4)[29], low for the other pollen allergens with only respiratory exposure and no cross-reactivity with food allergens (e.g., ragweed), and 3) for food allergens, IgG responses were frequent whereas IgE responses were rare. These results indicate that contact with allergen by the oral route may preferentially induce IgG responses whereas allergen contact by respiratory route appears important for IgE sensitization.

Strengths and weaknesses

One strength lies in the solid phenotypic characterization of a large population, with a fair distribution of asymptomatic subjects and subjects with asthma and/or rhinitis. As in most epidemiological settings, the definition of asthma and rhinitis was based on a standardized questionnaire, which might have lead to misclassification bias on the disease status, but should not have biased our results on the effects of the geographical area and routes of exposure in IgE and IgG recognition. As compared to the general population, our study population is enriched with subjects with asthma and consequently has higher percentages of IgE-sensitizations, leading to an increased statistical power to address allergen-specific IgE-responses. Indeed, since the main aim of the present manuscript was the parallel analysis of allergen-specific IgE and IgG responses towards a comprehensive set of allergen molecules to dissect variations in geographic sensitization

profiles and possible routes of sensitization, a normal population would not have been appropriate as the percentage of allergic subjects would have been too low and it would have required a much larger sample size. This population selection should not have affected our results on the geographical variability of IgE and IgG sensitisation, but does not allow assessing the community-based prevalence of IgE and IgG responses. The study is specific to France, but can help to understand and assess the differences between ubiquitous and regional allergens in other countries as well as IgE and IgG responses to allergens in general.

The effect of immunotherapy on specific-IgE or specific-IgG levels could not be tested since only allergic patients had received this form of treatment and there will necessarily be higher frequencies of IgE recognition in individuals who received AIT as compared to those who did not. However, the frequency of subject who had received AIT was similar across centres and even though AIT induces IgG it does not completely eliminate IgE reactivity to the micro-arrayed allergens and therefore does not affect the geographic IgE results. Although we do not have detailed information on AIT (as the targeted allergens) we do not think that AIT might differentially influence the IgE/IgG profile according to the allergen. For instance, it is unlikely that subjects in areas without birch pollen developed IgG against this allergen (i.e. Bet v 1 and Bet v 1-related food allergens) due to AIT because birch-pollen specific AIT was probably not prescribed in this area. Also, naturally occurring IgG does not affect IgE recognition in the chip analysis because naturally occurring IgG seems to react with epitopes different from those recognized by IgE [8]. Finally the sensitivity analysis conducted among subjects not having received AIT showed strong robustness of the observed associations.

The exposure to allergens was not addressed at the individual level, but by study centre locations. This ecological approach for exposure assessment might be a source of exposure misclassification, characterized here by the Berkson error type which has been shown to weaken the precision of the estimates, but as compared to the classical error does not induce major bias on risk estimates [30]. Also, we considered the center of recruitment and did not take into account the residential history of the participants. This may have lead to exposure misclassification because some individuals might have lived in different areas with potentially different exposures.

A major strength relates to the use of the improved microarray technology, which contains a larger number of important allergens than the commercial ISAC chips.[15,16] The MeDALL chip used in this study is as sensitive for IgE detection as the quantitative ImmunoCAP assay and can be used also for the detection of allergen-specific IgG responses with high specificity and sensitivity.[15,16] Furthermore, the allergen repertoire on the MeDALL chip covers most of the relevant allergen sources.[17] Although IgG can interfere with IgE binding on allergen-microarrays, we have proven that it does not affect the sensitivity to pick up sensitizations. [17] Moreover, we found that IgE results were in good agreement with results obtained by skin prick testing.

Interpretation of the results

In accordance with a recent cross-sectional analysis in the United States, IgE sensitisation to at least one of the studied allergens did not vary across the French regions but allergen-specific sensitisation against some allergen components exhibited geographical variations.[31] This may indicate that genetic susceptibility for sensitization, probably similar across the country, favors an IgE immune response in the same percentage of the population overall, but specificities of IgE reactions are linked to exposure. Indeed, chip testing reveals that the molecular IgE sensitization profile for respiratory allergens in France correlates very well with exposure data: some plants being prevalent in defined areas (birch, ragweed and cypress) and some allergen sources being found across France (grass, olive/ash pollen).[32-34] Patients with a subclinical sensitization to birch pollen exist in an area without atmospheric birch pollen exposure.[35] For cypress pollen, IgE patterns also reflect pollen dispersion but IgG is found in most participants because of the carbohydrate nature of the allergen which makes it a pan-allergen.[29] The lower IgE recognition frequency of Der p 1 and Der p 2 in Lyon was *a priori* not expected but might be related to factors like living habits.

Some respiratory allergens induce IgG only in few patients because they are poorly immunogenic (e.g., Phl p 2) but nevertheless they often lead to IgE-sensitization.[36,37] This follows properties of the antigens themselves. We found that IgG reactivity to the highly cross-reactive pollen allergens Phl p 12 and Bet v 2 (i.e., profilins) [38] and PR-10 proteins[39] was very common in each region whereas IgE and IgG reactivity to another class of cross-

reactive pollen allergens, the calcium-binding allergens from birch (Bet v 4) and timothy grass (Phl p 7) was low in all regions. PR-10 and profilins are present in pollen and plant-derived foods whereas the polcalcins, i.e., calcium-binding allergen components are present only in pollen. This probably explains the differences in IgG reactivity between PR-10 and profillins (high) by comparison to calcium-binding components (low). This is supported by a recent study suggesting that foodborne PR-10 allergens initiate an early IgG-response to PR-10 molecules but no IgE-response.[11] Certain house dust mite allergens that are rare targets for IgE (Der p 10, Der p 11 and Der p 14, < 10% in our population) are frequently recognized by IgG antibodies (>60% in our population). Interestingly, these allergens are mainly present in mite bodies and therefore allergen contact occurs mainly via the skin [40] or via cross-reactive food allergens (e.g., Der p 10 is cross-reactive with tropomyosin from shrimps).

In conclusion, the results from the analysis of IgE and IgG responses against a comprehensive set of respiratory and food allergens in the present study suggest that IgG response is related to exposure via the respiratory tract, the gastrointestinal tract and possibly via the skin whereas IgE sensitization occurs mainly when the subject is exposed via the respiratory tract. However, only a fraction of these subjects develops IgE antibodies.[18] The results of the present study will be valuable for better understanding when allergen-specific interventions like avoidance or immunotherapy should be undertaken or not.

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569

570 **TABLE I. Description of the study population (n=340)**

571

	n (%) / m (sd)
Number of males (%)	159 (46.8 %)
Mean age (±sd)	43.4 (±16.8)
Status at recruitment	
Asthma cases, n (%)	83 (24.4)
First-degree relatives of cases, n (%)	184 (54.1)
Controls, n (%)	73 (21.5)
Recruitment center	
Paris, n (%)	96 (28.2)
Lyon, n (%)	75 (22.1)
Marseille, n (%)	41 (12.0)
Montpellier, n (%)	22 (6.5)
Grenoble, n (%)	106 (31.2)
Asthma ever, n (%)	170 (50 %)
Asthma in the past 12 months, n (%)	138 (87.3 %)
Allergic sensitization (≥1 positive SPT among the 11 allergen sources)*, n (%)	197 (57.9%)
Total IgE > 100 IU/ml**, n (%)	145 (42.7 %)
Allergic rhinitis ever, n (%)	176 (52.4 %)
Allergic rhinitis in the past 12 months, n (%)	138 (44.8 %)
Blood eosinophil counts > 250 mm^3, n (%)	86 (25.4 %)
Atopic dermatitis ever, n (%)	127 (37.6 %)
FEV_1 < 80% of predicted value, (%)	46 (13.6 %)
Combined Asthma (ever) and allergic rhinitis (ever) phenotypes:	
No asthma and no rhinitis, n (%)	121 (31.0 %)
No asthma but rhinitis, n (%)	48 (14.3 %)
Asthma but no rhinitis, n (%)	39 (11.6 %)
Asthma and rhinitis, n (%)	128 (38.1 %)

572 *****11 allergen sources were : cat, *Dermatophagoides pteronyssinus*, *Blattella germanica*, olive, birch,
573 *Parietaria judaica*, timothy grass, ragweed, *Aspergillus*, *Cladosporium herbarum*, *Alternaria tenuis*
574 ** The threshold of 100 IU/ml has been used as in many other studies

575 **TABLE II. Allergen specific IgE and IgG description**

		In all (n=340)				In subjects without AIT* (n=258)			
		IgE ≥ 0.3 ISU		IgG ≥ 0.5 ISU		IgE ≥ 0.3 ISU		IgG ≥ 0.5 ISU	
Allergen-source	Allergen	n	%	n	%	n	%	n	%
Inhaled allergens									
Birch	rBet v 1	34	10.0	225	66.6	18	7.0	165	64.2
	rBet v 4	13	3.8	109	32.2	3	1.2	79	30.7
	rBet v 2	10	2.9	288	85.2	3	1.2	218	84.8
Olive	rOle e 1	71	20.9	220	65.1	39	15.1	157	61.1
	nOle e 7	5	1.5	67	19.8	2	0.8	46	17.9
	rOle e 9	2	0.6	338	100.0	1	0.4	257	100.0
Cypress	nCup a 1	62	18.2	255	75.4	32	12.4	193	75.1
Timothy Grass	rPhl p 1	115	33.8	322	95.3	67	26.0	244	94.9
	rPhl p 2	52	15.3	115	34.0	25	9.7	76	29.6
	nPhl p 4	76	22.4	282	83.4	35	13.6	209	81.3
	rPhl p 5	62	18.2	218	64.5	23	8.9	161	62.6
	rPhl p 6	49	14.4	229	67.8	20	7.8	169	65.8
	rPhl p 7	13	3.8	94	27.8	3	1.2	68	26.5
	rPhl p 11	23	6.8	133	39.3	7	2.7	93	36.2
	rPhl p 12	6	1.8	310	91.7			236	91.8
Ragweed	nAmb a 1	13	3.8	109	32.2	8	3.1	81	31.5
House dust mite	nDer p 1	83	24.4	317	93.8	43	16.7	238	92.6
	rDer p 2	93	27.4	278	82.2	49	19.0	206	80.2
	rDer p 4	54	15.9	142	42.0	27	10.5	102	39.7
	rDer p 5	56	16.5	321	95.0	23	8.9	246	95.7
	rDer p 7	45	13.2	301	89.1	16	6.2	228	88.7
	rDer p 10	31	9.1	214	63.3	13	5.0	156	60.7
	rDer p 11	15	4.4	331	97.9	6	2.3	251	97.7
	rDer p 14	7	2.1	334	98.8	2	0.8	254	98.8
	rDer p 15	15	4.4	327	96.7	6	2.3	249	96.9
	rDer p 18	17	5.0	328	97.0	4	1.6	250	97.3
	rDer p 21	34	10.0	334	98.8	14	5.4	255	99.2
	rDer p 23	93	27.4	335	99.1	50	19.4	255	99.2
	rclone 16	33	9.7	292	86.4	12	4.7	218	84.8
Cat	rFel d 1	81	23.8	306	90.5	41	15.9	233	90.7
	nFel d 2	25	7.4	179	53.0	12	4.7	130	50.6
	rFel d 4	18	5.3	253	74.9	6	2.3	192	74.7
Dog	rCan f 1	22	6.5	202	59.8	7	2.7	152	59.1
	rCan f 2	8	2.4	188	55.6	2	0.8	139	54.1
	nCan f 3	8	2.4	164	48.5	3	1.2	121	47.1
	rCan f 4	16	4.7	296	87.6	2	0.8	228	88.7
	rCan f 5	38	11.2	265	78.4	18	7.0	198	77.0
	rCan f 6	16	4.7	277	82.0	6	2.3	212	82.5
Food allergens									
Apple	rMal d 1	26	7.6	284	84.0	14	5.4	216	84.0
Hazelnut	rCor a 1.0401	24	7.1	234	69.2	12	4.7	176	68.5
Peanut	rAra h 8	16	4.7	233	68.9	6	2.3	180	70.0

Peach	rPru p 1	20	5.9	242	71.6	10	3.9	180	70.0
Soy	rGly m 4	8	2.4	292	86.4	2	0.8	221	86.0
Milk	nBos d 4	1	0.3	299	88.5	1	0.4	232	90.3
	nBos d 8	0	0.0	309	91.4			234	91.1
Egg	nGal d 1	2	0.6	289	85.5			217	84.4
Codfish	rGad c 1	0	0.0	95	28.1			72	28.0

576 * Allergen Immunotherapy

577 **TABLE III. Adjusted odds ratios* for different centers of recruitment for being IgE- or IgG-positive to different airborne allergens**
578

		IgE (<0.3; ≥0.3)					IgG (<0.5; ≥0.5)				
Allergen	Center	OR	Lower 95% CI	Upper 95% CI	p value	Global p value	OR	Lower 95% CI	Upper 95% CI	p value	Global p value
Amb a 1	Lyon	-					**3.56**	**1.81**	**7.02**	**<0.01**	**0.001**
	Marseille	-					**2.52**	**1.09**	**5.85**	**0.03**	
	Montpellier	-					0.79	0.23	2.67	0.70	
	Grenoble	-					1.32	0.69	2.51	0.40	
Bet v 1	Lyon	-					1.57	0.8	3.09	0.19	0.5
	Marseille	-					1.64	0.7	3.83	0.26	
	Montpellier	-					0.93	0.34	2.54	0.88	
	Grenoble	-					1.53	0.83	2.83	0.17	
Cup a 1	Lyon	0.67	0.24	1.84	0.44	**0.0009**	1.38	0.66	2.87	0.39	0.29
	Marseille	**3.56**	**1.17**	**10.9**	**0.03**		1.7	0.64	4.49	0.29	
	Montpellier	**7.37**	**2.15**	**25.3**	**<0.01**		**7.79**	**0.98**	**62.2**	**0.05**	
	Grenoble	0.85	0.36	1.96	0.7		1.05	0.56	1.96	0.88	
Phl p 1	Lyon	0.81	0.36	1.83	0.61	0.69	4.87	0.57	41.7	0.15	-
	Marseille	1.29	0.47	3.58	0.62		-				
	Montpellier	0.68	0.2	2.26	0.52		1.28	0.14	11.9	0.83	
	Grenoble	1.28	0.63	2.6	0.5		1.33	0.42	4.2	0.62	
Der p 1	Lyon	**0.37**	**0.15**	**0.96**	**0.04**	**0.01**	**13.6**	**1.68**	**110**	**0.01**	-
	Marseille	0.28	0.07	1.12	0.07		-				
	Montpellier	1.00	0.28	3.58	0.99		0.81	0.21	3.08	0.76	
	Grenoble	1.53	0.75	3.11	0.24		**4.22**	**1.28**	**13.9**	**0.02**	
Der p 2	Lyon	**0.32**	**0.13**	**0.81**	**0.02**	**0.004**	**2.48**	**1.05**	**5.85**	**0.04**	0.12
	Marseille	0.41	0.13	1.36	0.15		1.42	0.54	3.73	0.48	
	Montpellier	0.91	0.27	3.07	0.88		7.1	0.86	58.7	0.07	
	Grenoble	1.67	0.83	3.38	0.15		1.87	0.89	3.9	0.1	

*Odds Ratio (reference: Paris) adjusted for age, sex, asthma status and rhinitis status
Some ORs could not be estimated because of sample size (represented by - in the table)
ORs associated with a p value < 0.05 are presented in bold.

Figure legends

FIG 1. IgE and IgG recognition frequencies and intensities for genuine pollen marker allergens from (**A**) ragweed (Amb a 1), (**B**) birch (Bet v 1), (**C**) cypress (Cup a 1), (**D**) olive/ash (Ole e 1), (**E**) timothy grass (Phl p 1) and for cross-reactive pollen allergens (**F**) timothy grass polcalcin(Phl p 7), (**G**) birch polcalcin (Bet v 4), (H) timothy grass profilin (Phl p 12) and (**I**) birch profilin (Bet v 2), and for the Bet v 1-related food allergen from (**J**) apple (Mal d 1) in different regions of France. Shown are the percentages of subjects (y-axes) with IgE (upper panel) and IgG reactivity (middle panel) to the allergens in the different cities of France (x-axes) and the pollen counts in the different areas of France (lower panels). Antibody levels are colour-coded and shown in ISU (ISAC standardized units).
P values assessing the differences across centres were estimated from the non-parametric Kruskal-Wallis test.

FIG 2. IgE and IgG recognition frequencies and intensities for respiratory house dust mite allergens derived from mite feces, i.e., (**A**) Der p 1, (**B**) Der p 2, (**C**) Der p 23 and from mite bodies, i.e., (**D**) Der p 10, (**F**) Der p 11 and (**F**) Der p 14, respectively. Shown are the percentages of subjects (y-axes) with IgE (upper panel) and IgG reactivity (lower panel) to the allergens in the different cities of France (x-axes). Antibody levels are color-coded and shown in ISU.
P values assessing the differences across centers were estimated from the non-parametric Kruskal-Wallis test.

FIG 3. IgG recognition frequencies and intensities for classical food allergens derived from (**A**) milk (Bos d 4), (**B**) egg (Gal d 1) and (**C**) fish (Gad c 1). Shown are the percentages of subjects (y-axes) with IgG reactivity to the allergens in the different cities of France (x-axes). Antibody levels are color-coded and shown in ISU.
P values assessing the differences across centers were estimated from the non-parametric Kruskal-Wallis test.

Online repository

Specific IgE and IgG measured by the MeDALL allergen-chip depend on allergen and route of exposure - the EGEA study

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TABLE EI. Comparison of included and non-included subjects

	Subjects without asthma			Subjects with asthma		
	Non-Included	Included	P value	Non-Included	Included	P value
n	697	170		507	170	
Age, m (sd)	46.3 (16.0)	46.8 (16.7)	0.68	38.8 (16.8)	40.0 (16.4)	0.42
Sex, %males	46.6	47.7	0.81	49.1	41.2	0.07
Center of recruitment			0.51			0.02
Paris, %	26.7	27.7		35.1	28.8	
Lyon, %	17.9	22.9		13.0	21.2	
Marseille, %	17.8	14.1		16.4	10.0	
Montpellier, %	7.0	5.9		8.1	7.1	
Grenoble, %	30.6	29.4		27.4	32.9	
Cat ownership [1], %	53.2	48.2	0.24	49.1	52.9	0.39
Allergic rhinitis ever, %	30.2	28.4	0.65	70.1	76.7	0.10
Allergic sensitization (≥1SPT among 12 allergens), %	38.9	34.7	0.33	76.3	81.2	0.20
Total IgE>100IU/ml, %	30.7	24.7	0.13	59.2	60.6	0.75

[1] current cat ownership or in childhood

TABLE E2. Agreement between SPT and allergen specific-IgE for those 7 allergen sources that showed a prevalence for positive SPT > 5% in the study population.

	number of components on the MeDALL chip, n	positive SPT, %	Allergen specific-IgE ≥0.3 for at least one of the allergen components, %	Cohen Kappa coefficient
Timothy Grass	8	33.5	39.4	0.76
House dust mite	15	33.5	34.7	0.83
Cat	3	21.8	23.8	0.76
Olive	3	20.6	21.2	0.79
Birch	3	10.6	13.8	0.71
Alternaria	2	8.8	9.4	0.65
Ragweed	1	5.9	3.8	0.71

TABLE E3. Unadjusted odds ratios for different centers of recruitment for being IgE- or IgG-positive to different airborne allergens (reference: Paris), by asthma status

		In subjects without asthma (n=170)								In subjects with asthma (n=170)							
		IgE (<0.3; ≥0.3)				IgG (<0.5; ≥0.5)				IgE (<0.3; ≥0.3)				IgG (<0.5; ≥0.5)			
Allergen	Center	OR	Lower 95% CI	Upper 95% CI	p value	OR	Lower 95% CI	Upper 95% CI	p value	OR	Lower 95% CI	Upper 95% CI	p value	OR	Lower 95%CI	Upper 95% CI	p value
Amb a 1	Lyon	-				2.25	0.91	5.61	0.08	-				5.46	2.09	14.3	<0.01
	Marseille	-				1.46	0.5	4.26	0.49	-				3.9	1.17	13	0.03
	Montpellier	-				1.25	0.28	5.62	0.77	-				0.35	0.04	3.08	0.35
	Grenoble	-				1.17	0.47	2.88	0.74	-				1.43	0.57	3.55	0.45
Bet v 1	Lyon	-				1.57	0.66	3.75	0.31	-				1.08	0.4	2.9	0.87
	Marseille	-				1.23	0.46	3.33	0.68	-				0.79	0.23	2.73	0.71
	Montpellier	-				0.88	0.22	3.45	0.85	-				0.72	0.19	2.81	0.64
	Grenoble	-				1.66	0.73	3.77	0.23	-				1.08	0.45	2.6	0.86
Cup a 1	Lyon	0.59	0.05	6.79	0.67	2.36	0.85	6.52	0.1	0.55	0.2	1.52	0.25	0.97	0.36	2.64	0.96
	Marseille	3.21	0.5	20.7	0.22	1.96	0.62	6.23	0.25	2.55	0.82	7.89	0.1	2.27	0.45	11.5	0.32
	Montpellier	15	2.25	100	0.01	2.06	0.39	10.9	0.39	2.27	0.63	8.19	0.21	-			
	Grenoble	0.94	0.13	6.94	0.95	1.43	0.6	3.43	0.42	0.69	0.29	1.63	0.39	0.74	0.31	1.77	0.5
Phl p 1	Lyon	0.56	0.15	2.01	0.37	2.59	0.26	26	0.42	0.88	0.37	2.09	0.78	-			
	Marseille	0.98	0.26	3.63	0.97	-				1	0.33	3.01	0.99	-			
	Montpellier	3.25	0.74	14.2	0.12	0.61	0.06	6.59	0.69	0.18	0.04	0.89	0.04	0.98	0.1	9.64	0.98
	Grenoble	1.22	0.44	3.41	0.71	1.6	0.26	10	0.61	1.02	0.47	2.2	0.96	1.16	0.27	4.89	0.84
Der p 1	Lyon	0.45	0.08	2.48	0.36	7.79	0.93	65.4	0.06	0.32	0.12	0.85	0.02	-			
	Marseille	-				-				0.35	0.1	1.22	0.1	-			
	Montpellier	0.93	0.1	8.99	0.95	1.85	0.2	16.7	0.59	0.57	0.15	2.13	0.4	0.27	0.05	1.4	0.12
	Grenoble	0.73	0.18	2.9	0.66	4.82	0.97	24	0.06	1.51	0.7	3.26	0.3	2.4	0.42	13.7	0.32
Der p 2	Lyon	0.45	0.08	2.48	0.36	2.14	0.77	5.96	0.14	0.27	0.1	0.72	0.01	2.15	0.53	8.74	0.29
	Marseille	0.76	0.14	4.26	0.76	1.14	0.39	3.33	0.81	0.4	0.12	1.31	0.13	1.37	0.26	7.21	0.71
	Montpellier	0.93	0.1	8.99	0.95	-				0.69	0.19	2.46	0.56	2.15	0.24	19	0.49
	Grenoble	1.15	0.32	4.04	0.83	1.62	0.65	4.02	0.3	1.48	0.68	3.22	0.32	1.63	0.52	5.06	0.4

TABLE E4. Adjusted odds ratios for different centers of recruitment for being IgE- or IgG-positive to different allergens (reference: Paris) among subjects without allergen immunotherapy

		IgE (<0.3; ≥0.3)				IgG (<0.5; ≥0.5)			
Allergen	Center	OR	Lower 95% CI	Upper 95% CI	p value	OR	Lower 95% CI	Upper 95% CI	p value
Amb a 1	Lyon	-				**2.60**	**1.20**	**5.65**	**0.02**
	Marseille	-				1.92	0.73	5.03	0.18
	Montpellier	-				0.79	0.19	3.25	0.74
	Grenoble	-				1.15	0.55	2.42	0.72
Bet v 1	Lyon	0.42	0.08	2.19	0.30	1.77	0.81	3.85	0.15
	Marseille	-			0.95	1.60	0.62	4.13	0.34
	Montpellier	-			0.97	0.50	0.15	1.64	0.25
	Grenoble	0.87	0.25	2.98	0.82	1.34	0.67	2.69	0.40
Cup a 1	Lyon	0.51	0.10	2.58	0.42	1.35	0.58	3.16	0.48
	Marseille	**4.64**	**0.99**	**21.70**	**0.05**	1.71	0.55	5.36	0.36
	Montpellier	**13.79**	**2.64**	**72.02**	**0.00**	5.32	0.64	44.38	0.12
	Grenoble	1.33	0.39	4.54	0.65	0.84	0.41	1.73	0.64
Phl p 1	Lyon	0.74	0.26	2.12	0.57	3.46	0.37	32.18	0.28
	Marseille	2.17	0.61	7.67	0.23	-			
	Montpellier	1.24	0.28	5.45	0.78	0.76	0.07	7.86	0.82
	Grenoble	1.17	0.48	2.85	0.72	1.17	0.31	4.33	0.82
Der p 1	Lyon	0.64	0.19	2.12	0.46	11.25	1.37	92.65	0.02
	Marseille	0.16	0.02	1.50	0.11	-			
	Montpellier	0.45	0.05	4.29	0.48	0.57	0.14	2.34	0.44
	Grenoble	1.61	0.64	4.10	0.31	**3.39**	**1.00**	**11.47**	**0.05**
Der p 2	Lyon	0.53	0.16	1.71	0.29	2.31	0.93	5.78	0.07
	Marseille	0.49	0.11	2.28	0.36	1.57	0.53	4.63	0.41
	Montpellier	0.68	0.12	4.01	0.67	5.23	0.62	44.38	0.13
	Grenoble	2.07	0.83	5.17	0.12	1.98	0.89	4.40	0.10

*Odds Ratio (reference: Paris) adjusted for age, sex, asthma status and rhinitis status
Some ORs could not be estimated because of sample size (represented by - in the table)
ORs associated with a p value≤0.05 are presented in bold.

E-FIGURE Legend

FIG E1. IgE and IgG recognition frequencies and intensities in subjects not reported allergen immunotherapy for (A) ragweed (Am b a1), (B) birch (Bet v 1), (C) Cypress (cup a 1), (D) timothy grass (Phl p 1), (E) houst dust mit (Der p 1), (F) house dust mite (Der p 10) and (G) apple (Mal d 1)