



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

Valérie Siroux, Christian Lupinek, Yvonne Resch, Mirela Curin, Jocelyne Just, Thomas Keil, Renata Kiss, Karin Lodrup Carlsen, Erik Melen, Rachel Nadif, et al.

► To cite this version:

Valérie Siroux, Christian Lupinek, Yvonne Resch, Mirela Curin, Jocelyne Just, et al.. Specific IgE and IgG measured by the MeDALL allergen-chip depend on allergen and route of exposure: The EGEA study. *Journal of Allergy and Clinical Immunology*, 2017, 139 (2), pp.643 - 654. 10.1016/j.jaci.2016.05.023 . inserm-01815134

HAL Id: inserm-01815134

<https://inserm.hal.science/inserm-01815134v1>

Submitted on 13 Jun 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

Valérie Siroux^{a,b,c}, Christian Lupinek^d, Yvonne Resch^d, Mirela Curin^d, Jocelyne Just^{e, f},
Thomas Keil^{g, h}, Renata Kiss^d, Karin Lodrup Carlsen^{i, j}, Erik Melén^{k, l}, Rachel Nadif^{m, n},
Isabelle Pin^{a, b, c}, Ingebjørg Skrindo^{j, o}, Susanne Vrtala^{d, p}, Magnus Wickman^{l, m}, Josep Maria
Anto^{q, r, s, t}, Rudolf Valenta^d, Jean Bousquet^{m, n, u}

^a Univ. Grenoble Alpes. IAB. Team of Environmental Epidemiology applied to
Reproduction and Respiratory Health. F-38000 Grenoble. France.

^b INSERM. IAB. Team of Environmental Epidemiology applied to Reproduction and
Respiratory Health. F-38000 Grenoble. France.

^c CHU de Grenoble. IAB. Team of Environmental Epidemiology applied to Reproduction
and Respiratory Health. F-38000 Grenoble. France.

^d Division of Immunopathology, Department of Pathophysiology and Allergy Research,
Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna,
Austria.

^e Assistance Publique-Hôpitaux de Paris, Hôpital Armand-Trousseau, Allergology
Department, Paris, France

^f Université Paris 6 Pierre et Marie Curie, Paris, France

^g Institute for Social Medicine, Epidemiology and Health Economics, Charité –
Universitätsmedizin Berlin, Berlin, Germany

^h Institute for Clinical Epidemiology and Biometry, University of Würzburg, Würzburg,
Germany

ⁱ Dept of Paediatrics, Oslo University Hospital, Oslo, Norway

^j Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, Oslo, Norway

^k Institute of Environmental Medicine and the Centre for Allergy Research, Karolinska Institutet, Stockholm, Sweden

^l Sachs Children and Youth Hospital, Stockholm, Sweden

^m INSERM U1168, VIMA (Aging and chronic diseases. Epidemiological and public health approaches), 16 avenue Paul Vaillant Couturier, F-94807, Villejuif, France

ⁿ Univ Versailles St-Quentin-en-Yvelines, UMR-S 1168, F-78180, Montigny le Bretonneux, France

^o Department of Otorhinolaryngology, Akershus University Hospital, Norway

^p Christian Doppler Laboratory for the Development of Allergen chips, Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Austria.

^q Centre for Research in Environmental Epidemiology (CREAL)

^r IMIM (Hospital del Mar Medical Research Institute)

^s Universitat Pompeu Fabra (UPF)

^t CIBER Epidemiología y Salud Pública (CIBERESP)

^u University hospital, Montpellier, France and MeDALL (Mechanisms of the Development of Allergy, FP7)

Disclosure of potential conflict of interest related to the submitted work

Rudolf Valenta has received research grants from Biomay AG, Vienna, Austria and Thermofisher, Uppsala, Sweden. He serves as a consultant for Biomay AG, Vienna, Austria, Thermofisher, Uppsala, Sweden and Fresenius Medical Care, Bad Homburg, Germany. Jean Bousquet serves in scientific advisory boards from Almirall, Meda, Merck, MSD, Novartis, Sanofi-Aventis, Takeda, Teva, Uriach. Magnus Wickman has received research grants from ThermoFisher Scientific.

Corresponding author

54 Valérie Siroux
55 Institut Albert Boniot
56 Inserm/CNRS/Univ Grenoble-Alpes U 1209
57 Equipe d'épidémiologie environnementale appliquée à la reproduction et à la santé
58 respiratoire
59 Rond point de la chantourne
60 38706 La Tronche cedex
61 Tel : +33 4 76 54 95 56
62 Mail : Valerie.siroux@ujf-grenoble.fr

63

64 **Sources of funding:** The study was supported in part by Inserm Aviesan Itmo santé
65 publique, the Scientific committee "AGIR for chronic diseases", grant F4605 of the
66 Austrian Science Fund (FWF) to RV and by the European Commission's Seventh
67 Framework 29 Program MeDALL under grant agreement no. 261357.

68

69

70 **Manuscript word count:** 3605

71 **Abstract word count:** 250

72

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 allergens¹ 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 hyperresponsiveness and atopy - EGEA¹⁹⁻²¹ 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*, *Blattella*

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

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/m³ 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, respectively¹⁶⁻¹⁸. 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. Chi² 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 were 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 ($\geq 20\%$ subjects) were frequently recognized by IgG as well ($\geq 60\%$ subjects). Likewise, some respiratory allergens less frequently

recognized by IgE antibodies ($\leq 6\%$ 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).

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

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

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 IgE⁸. 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 estimates³⁰. 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 proteins³⁹ 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 profilins (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 skin⁴⁰ 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.

Acknowledgments

The authors thanks the EGEA cooperative group:

Coordination : V Siroux (epidemiology, PI since 2013); F Demenais (genetics); I Pin (clinical aspects); R Nadif (biology); F Kauffmann (PI 1992-2012).

Respiratory epidemiology : *Inserm U 700, Paris* : M Korobaëff (Egea1), F Neukirch (Egea1); *Inserm U 707, Paris* : I Annesi-Maesano (Egea1-2) ; *Inserm CESP/U 1018, Villejuif* : F Kauffmann, N Le Moual, R Nadif, MP Oryszczyn (Egea1-2), R Varraso ; *Inserm U 823, Grenoble* : V Siroux.

Genetics : *Inserm U 393, Paris* : J Feingold ; *Inserm U 946, Paris* : E Bouzigon, F Demenais, MH Dizier ; *CNG, Evry* : I Gut (now CNAG, Barcelona, Spain), M Lathrop (now Univ McGill, Montreal, Canada).

Clinical centers : *Grenoble* : I Pin, C Pison; *Lyon* : D Ecochard (Egea1), F Gormand, Y Pacheco ; *Marseille* : D Charpin (Egea1), D Vervloet (Egea1-2) ; *Montpellier* : J Bousquet ; *Paris Cochin* : A Lockhart (Egea1), R Matran (now in Lille) ; *Paris Necker* : E Paty (Egea1-2), P Scheinmann (Egea1-2) ; *Paris-Trousseau* : A Grimfeld (Egea1-2), J Just.

Data and quality management : *Inserm ex-U155 (Egea1)* : J Hochez ; *Inserm CESP/U 1018, Villejuif* : N Le Moual ; *Inserm ex-U780* : C Ravault (Egea1-2) ; *Inserm ex-U794* : N Chateigner (Egea1-2) ; *Grenoble* : J Quentin-Ferran (Egea1-2).

The authors thank all those who participated in the setting of the study and in the various aspects of the examinations involved: interviewers; technicians for lung function testing, skin prick tests, and IgE determinations; coders; those involved in quality control and data and sample management; and all those who supervised the study in all centers. They are also grateful to the biobanks in Lille (CIC-Inserm), and Annemasse (Etablissement français du sang) where biological samples are stored. They are indebted to all the individuals who participated, without whom the study would not have been possible.

The authors thanks the RNSA (Réseau National de Surveillance Aérobiologique), the French aerobiology network in charge of the analysis of biological particles in air samples, for providing access to the data of pollen concentrations across France.

The authors thank Rob Aalberse, Amsterdam, The Netherlands for thoughtful discussions.

454 The study was supported in part by Inserm Aviesan Itmo santé publique, the Scientific
455 committee “AGIR for chronic diseases”, grant F4605 of the Austrian Science Fund (FWF)
456 to RV and by the European Commission’s Seventh Framework 29 Program MeDALL under
457 grant agreement no. 261357.

458

459 **References**

- 460 1. Valenta R. The future of antigen-specific immunotherapy of allergy. *Nat Rev*
461 *Immunol* 2002;2:446-53.
- 462 2. Jenmalm MC, Bjorksten B. Development of immunoglobulin G subclass antibodies to
463 ovalbumin, birch and cat during the first eight years of life in atopic and non-atopic
464 children. *Pediatr Allergy Immunol* 1999;10:112-21.
- 465 3. Hofmaier S, Comberiati P, Matricardi PM. Immunoglobulin G in IgE-mediated allergy
466 and allergen-specific immunotherapy. *Eur Ann Allergy Clin Immunol* 2014;46:6-11.
- 467 4. Matsuoka T, Shamji MH, Durham SR. Allergen immunotherapy and tolerance.
468 *Allergol Int* 2013;62:403-13.
- 469 5. Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy: multiple
470 suppressor factors at work in immune tolerance to allergens. *J Allergy Clin Immunol*
471 2014;133:621-31.
- 472 6. Larche M, Akdis CA, Valenta R. Immunological mechanisms of allergen-specific
473 immunotherapy. *Nat Rev Immunol* 2006;6:761-71.
- 474 7. Braza F, Chesne J, Castagnet S, Magnan A, Brouard S. Regulatory functions of B cells
475 in allergic diseases. *Allergy* 2014;69:1454-63.
- 476 8. Curin M, Swoboda I, Wollmann E, et al. Microarrayed dog, cat, and horse allergens
477 show weak correlation between allergen-specific IgE and IgG responses. *J Allergy*
478 *Clin Immunol* 2014;133:918-21 e6.
- 479 9. Lau S, Illi S, Platts-Mills TA, et al. Longitudinal study on the relationship between cat
480 allergen and endotoxin exposure, sensitization, cat-specific IgG and development of
481 asthma in childhood--report of the German Multicentre Allergy Study (MAS 90).
482 *Allergy* 2005;60:766-73.
- 483 10. Erwin EA, Woodfolk JA, James HR, Satinover SM, Platts-Mills TA. Changes in cat
484 specific IgE and IgG antibodies with decreased cat exposure. *Ann Allergy Asthma*
485 *Immunol* 2014;112:545-50 e1.
- 486 11. Hofmaier S, Hatzler L, Rohrbach A, et al. "Default" versus "pre-atopic" IgG responses
487 to foodborne and airborne pathogenesis-related group 10 protein molecules in

488 birch-sensitized and nonatopic children. *J Allergy Clin Immunol* 2015;135:1367-74
489 e8.

490 12. Hiller R, Laffer S, Harwanegg C, et al. Microarrayed allergen molecules: diagnostic
491 gatekeepers for allergy treatment. *FASEB J* 2002;16:414-6.

492 13. Canonica GW, Ansotegui IJ, Pawankar R, et al. A WAO - ARIA - GA(2)LEN consensus
493 document on molecular-based allergy diagnostics. *World Allergy Organ J* 2013;6:17.

494 14. Melioli G, Bonifazi F, Bonini S, et al. The ImmunoCAP ISAC molecular allergology
495 approach in adult multi-sensitized Italian patients with respiratory symptoms. *Clin*
496 *Biochem* 2011;44:1005-11.

497 15. Bousquet J, Anto J, Auffray C, et al. MeDALL (Mechanisms of the Development of
498 ALLergy): an integrated approach from phenotypes to systems medicine. *Allergy*
499 2011;66:596-604.

500 16. Lupinek C, Wollmann E, Baar A, et al. Advances in allergen-microarray technology
501 for diagnosis and monitoring of allergy: the MeDALL allergen-chip. *Methods*
502 2014;66:106-19.

503 17. Skrindo I, Lupinek C, Valenta R, et al. The use of the MeDALL-chip to assess IgE
504 sensitization: a new diagnostic tool for allergic disease? *Pediatr Allergy Immunol*
505 2015;26:239-46.

506 18. Westman M, Lupinek C, Bousquet J, et al. Early childhood IgE reactivity to
507 pathogenesis-related class 10 proteins predicts allergic rhinitis in adolescence. *J*
508 *Allergy Clin Immunol* 2015;135:1199-206 e1-11.

509 19. Kauffmann F, Dizier MH, Annesi-Maesano I, et al. EGEA (Epidemiological study on
510 the Genetics and Environment of Asthma, bronchial hyperresponsiveness and
511 atopy)-- descriptive characteristics. *Clin Exp Allergy* 1999;29 Suppl 4:17-21.

512 20. Boudier A, Curjuric I, Basagana X, et al. Ten-year follow-up of cluster-based asthma
513 phenotypes in adults. A pooled analysis of three cohorts. *Am J Respir Crit Care Med*
514 2013;188:550-60.

515 21. Siroux V, Boudier A, Bousquet J, et al. Phenotypic determinants of uncontrolled
516 asthma. *J Allergy Clin Immunol* 2009;124:681-7.

- 517 22. Kauffmann F, Dizier MH. EGEA (Epidemiological study on the Genetics and
518 Environment of Asthma, bronchial hyperresponsiveness and atopy)--design issues.
519 EGEA Co-operative Group. Clin Exp Allergy 1995;25 Suppl 2:19-22.
- 520 23. Kauffmann F, Dizier MH, Pin I, et al. Epidemiological study of the genetics and
521 environment of asthma, bronchial hyperresponsiveness, and atopy: phenotype
522 issues. Am J Respir Crit Care Med 1997;156:S123-S9.
- 523 24. Wollmann E, Hamsten C, Sibanda E, et al. Natural clinical tolerance to peanut in
524 African patients is caused by poor allergenic activity of peanut IgE. Allergy 2015.
- 525 25. Thibaudon M. [The pollen-associated allergic risk in France]. Allerg Immunol (Paris)
526 2003;35:170-2.
- 527 26. Thibaudon M, Caillier J. Développement temporel et géographique des allergènes
528 polliniques principaux en France: 1987-2001. Allerg Immunol (Paris) 2002;34:154-
529 7.
- 530 27. Hatzler L, Panetta V, Lau S, et al. Molecular spreading and predictive value of
531 preclinical IgE response to Phleum pratense in children with hay fever. J Allergy Clin
532 Immunol 2012;130:894-901 e5.
- 533 28. Panzner P, Vachova M, Vitovcova P, Brodska P, Vlas T. A comprehensive analysis of
534 middle-European molecular sensitization profiles to pollen allergens. Int Arch
535 Allergy Immunol 2014;164:74-82.
- 536 29. Douladiris N, Savvatanos S, Roumpedaki I, Skevaki C, Mitsias D, Papadopoulos NG. A
537 molecular diagnostic algorithm to guide pollen immunotherapy in southern Europe:
538 towards component-resolved management of allergic diseases. Int Arch Allergy
539 Immunol 2013;162:163-72.
- 540 30. Armstrong BG. Effect of measurement error on epidemiological studies of
541 environmental and occupational exposures. Occup Environ Med 1998;55:651-6.
- 542 31. Salo PM, Arbes SJ, Jr., Jaramillo R, et al. Prevalence of allergic sensitization in the
543 United States: results from the National Health and Nutrition Examination Survey
544 (NHANES) 2005-2006. J Allergy Clin Immunol 2014;134:350-9.
- 545 32. Bousquet J, Guerin B, Hewitt B, Lim S, Michel FB. Allergy in the Mediterranean area.
546 III: Cross reactivity among Oleaceae pollens. Clin Allergy 1985;15:439-48.

- 547 33. Palomares O, Swoboda I, Villalba M, et al. The major allergen of olive pollen Ole e 1 is
548 a diagnostic marker for sensitization to Oleaceae. *Int Arch Allergy Immunol*
549 2006;141:110-8.
- 550 34. Castro L, Mas S, Barderas R, et al. Sal k 5, a member of the widespread Ole e 1-like
551 protein family, is a new allergen of Russian thistle (*Salsola kali*) pollen. *Int Arch*
552 *Allergy Immunol* 2014;163:142-53.
- 553 35. Yamagiwa M, Hattori R, Ito Y, et al. Birch-pollen sensitization in an area without
554 atmospheric birch pollens. *Auris Nasus Larynx* 2002;29:261-6.
- 555 36. Heiss S, Mahler V, Steiner R, et al. Component-resolved diagnosis (CRD) of type I
556 allergy with recombinant grass and tree pollen allergens by skin testing. *J Invest*
557 *Dermatol* 1999;113:830-7.
- 558 37. Vrtala S, Ball T, Spitzauer S, et al. Immunization with purified natural and
559 recombinant allergens induces mouse IgG1 antibodies that recognize similar
560 epitopes as human IgE and inhibit the human IgE-allergen interaction and allergen-
561 induced basophil degranulation. *J Immunol* 1998;160:6137-44.
- 562 38. Valenta R, Duchene M, Ebner C, et al. Profilins constitute a novel family of functional
563 plant pan-allergens. *J Exp Med* 1992;175:377-85.
- 564 39. Ebner C, Hirschwehr R, Bauer L, et al. Identification of allergens in fruits and
565 vegetables: IgE cross-reactivities with the important birch pollen allergens Bet v 1
566 and Bet v 2 (birch profilin). *J Allergy Clin Immunol* 1995;95:962-9.
- 567 40. Banerjee S, Resch Y, Chen KW, et al. Der p 11 is a major allergen for house dust mite-
568 allergic patients suffering from atopic dermatitis. *J Invest Dermatol* 2015;135:102-9.
569

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

	n (%) / m (sd)
Number of males (%)	159 (46.8 %)
Mean age (\pm sd)	43.4 (\pm 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 (\geq 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 %)

*11 allergen sources were : cat, *Dermatophagoides pteronyssinus*, *Blattella germanica*, olive, birch, *Parietaria judaica*, timothy grass, ragweed, *Aspergillus*, *Cladosporium herbarum*, *Alternaria tenuis*

** 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	11	33.8						
		5		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	24	7.1						
	1.0401			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

* Allergen Immunotherapy

576

577
578

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

Allergen	Center	IgE (<0.3; ≥0.3)					IgG (<0.5; ≥0.5)				
		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	

579 *Odds Ratio (reference: Paris) adjusted for age, sex, asthma status and rhinitis status
580 Some ORs could not be estimated because of sample size (represented by - in the table)
581 ORs associated with a p value < 0.05 are presented in bold.
582

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, (E) 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**

Valérie Siroux^{a,b,c}, Christian Lupinek^d, Yvonne Resch^d, Mirela Curin^d, Jocelyne Just^{e,f},
Thomas Keil^{g,h}, Renata Kiss^d, Karin Lodrup Carlsen^{i,j}, Erik Melén^{k,l}, Rachel Nadif^{m,n},
Isabelle Pin^{a,b,c}, Ingebjørg Skrindo^{j,o}, Susanne Vrtala^{d,p}, Magnus Wickman^{l,m}, Josep Maria
Anto^{q,r,s,t}, Rudolf Valenta^d, Jean Bousquet^{m,n,u}

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 (≥ 1 SPT 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
<i>Alternaria</i>	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

Allergen	Center	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)			
		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

Allergen	Center	IgE (<0.3; ≥0.3)				IgG (<0.5; ≥0.5)			
		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)