



**HAL**  
open science

## 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-01815134>**

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.

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

3

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

8

9 <sup>a</sup> Univ. Grenoble Alpes. IAB. Team of Environmental Epidemiology applied to  
10 Reproduction and Respiratory Health. F-38000 Grenoble. France.

11 <sup>b</sup> INSERM. IAB. Team of Environmental Epidemiology applied to Reproduction and  
12 Respiratory Health. F-38000 Grenoble. France.

13 <sup>c</sup> CHU de Grenoble. IAB. Team of Environmental Epidemiology applied to Reproduction  
14 and Respiratory Health. F-38000 Grenoble. France.

15 <sup>d</sup> Division of Immunopathology, Department of Pathophysiology and Allergy Research,  
16 Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna,  
17 Austria.

18 <sup>e</sup> Assistance Publique-Hôpitaux de Paris, Hôpital Armand-Trousseau, Allergology  
19 Department, Paris, France

20 <sup>f</sup> Université Paris 6 Pierre et Marie Curie, Paris, France

21 <sup>g</sup> Institute for Social Medicine, Epidemiology and Health Economics, Charité –  
22 Universitätsmedizin Berlin, Berlin, Germany

23 <sup>h</sup> Institute for Clinical Epidemiology and Biometry, University of Würzburg, Würzburg,  
24 Germany

25 <sup>i</sup> Dept of Paediatrics, Oslo University Hospital, Oslo, Norway

26 <sup>j</sup> Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, Oslo, Norway

27 <sup>k</sup> Institute of Environmental Medicine and the Centre for Allergy Research, Karolinska  
28 Institutet, Stockholm, Sweden

29 <sup>l</sup> Sachs Children and Youth Hospital, Stockholm, Sweden

30 <sup>m</sup> INSERM U1168, VIMA (Aging and chronic diseases. Epidemiological and public health  
31 approaches), 16 avenue Paul Vaillant Couturier, F-94807, Villejuif, France

32 <sup>n</sup> Univ Versailles St-Quentin-en-Yvelines, UMR-S 1168, F-78180, Montigny le Bretonneux,  
33 France

34 <sup>o</sup> Department of Otorhinolaryngology, Akershus University Hospital, Norway

35 <sup>p</sup> Christian Doppler Laboratory for the Development of Allergen chips, Department of  
36 Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and  
37 Immunology, Medical University of Vienna, Austria.

38 <sup>q</sup> Centre for Research in Environmental Epidemiology (CREAL)

39 <sup>r</sup> IMIM (Hospital del Mar Medical Research Institute)

40 <sup>s</sup> Universitat Pompeu Fabra (UPF)

41 <sup>t</sup> CIBER Epidemiología y Salud Pública (CIBERESP)

42 <sup>u</sup> University hospital, Montpellier, France and MeDALL (Mechanisms of the Development  
43 of Allergy, FP7)

44

#### 45 **Disclosure of potential conflict of interest related to the submitted work**

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

52

#### 53 **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](mailto: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

## 73 **Abstract**

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

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

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

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

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

96

## 97 **Key messages**

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

101

102 **Capsule summary**

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

107

108 **Key words**

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

111

112 **Abbreviations**

113 Bet v: *Betula verrucosa*

114 Cyn d: *Cynodon dactylon*

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

117 Fel d: *Felis domesticus*

118 FP7: Framework Programme 7 (European Union)

119 IgE: Immunoglobulin E

120 IgG: Immunoglobulin G

121 Mal d: *Malus domestica*

122 MeDALL: Mechanisms of the development of allergy

123 Phl p: *Phleum pratense*

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

## 125 **Introduction**

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

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

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

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

152 As part of the MeDALL project,<sup>15</sup> the present study investigates the geographical  
153 variability of allergen-specific IgE and IgG towards a large variety of different respiratory  
154 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



## 159 **Methods**

### 160 ***Population and study setting***

161 The EGEA cohort is composed of patients with asthma enrolled in chest clinics and their  
162 first-degree relatives, and a group of control subjects, all recruited in the early 1990s in 5  
163 French cities. Protocol and characteristics have been previously published.<sup>22,23</sup> Briefly,  
164 2047 participants were enrolled at baseline (EGEA1) including 388 children (<16 years)  
165 and adult patients with asthma from chest clinics, their 1244 first-degree relatives and  
166 415 population-based control subjects. Subjects were recruited through self-completed  
167 questionnaires and an overall matching by months at exam, age (decade), sex and centre.  
168 About 11 years later, this population was invited for a follow-up (EGEA2) and 1601  
169 participants (77.1% of the original cohort + 58 new family members) were subjected to a  
170 complete examination, which included serum samples.<sup>21</sup> EGEA collection is certified ISO  
171 9001 and referenced in the Biobank network by the number BB-0033-00043.

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

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

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

181 The definition of ever asthma was based on a positive answer to either “Have you ever  
182 had attacks of breathlessness at rest with wheezing?” or “Have you ever had asthma  
183 attacks?” or being recruited as an asthma case.<sup>23</sup> Allergic rhinitis ever was defined by a  
184 positive answer to “Have you ever had allergic rhinitis?” or “Have you ever had hay  
185 fever?”. Subjects with a positive answer to “Have you had a problem with sneezing or  
186 runny or blocked nose when you did not have a cold or flu in the last 12 months” have  
187 been defined as active allergic rhinitis.

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

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

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

193 IgE and IgG were determined in anonymized samples with the MeDALL-chip as  
194 described.<sup>15,16</sup> It comprises 176 allergen components including aero- and food allergen  
195 components. The measurement of allergen-specific IgE- and IgG reactivities was  
196 performed as described.<sup>16,18,24</sup>

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

### 206 **Geographical exposure to allergens**

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

### 212 **Biases**

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

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

221 ***Statistical methods***

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

231

232

## 233 **Results**

### 234 ***Characteristics of study subjects***

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

### 241 ***Most frequently recognized allergens by IgE***

242 Overall, 48.8% of the population had allergen-specific IgE  $\geq 0.3$  to at least one of the  
243 allergen components selected, which is in strong agreement with the prevalence of atopy  
244 (defined by positive SPT to at least one of 11 aeroallergens) in this population (Cohen  
245 Kappa coefficient = 0.68). The agreement between SPT and allergen-specific IgE was  
246 strong for each allergen (table E2). Pollen allergens most frequently recognized by IgE  
247 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%),  
248 Phl p 2 (15.3%), Phl p 6 (14.4%) and Bet v 1 (10%) (Table 2). The indoor allergens most  
249 frequently recognized by IgE were Der p 2 (27.4%), Der p 23 (27.4%), Der p 1 (24.4%), Fel  
250 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  
251 (10%) (Table 2). Regarding food allergens, the family of PR-10 proteins (i.e., Mal d 1:  
252 apple; Cor a 1.0401: hazelnut; Ara h 8: peanut; Pru p 1: cherry; Gly m 4: soy) that cross-  
253 react with the major birch pollen allergen Bet v 1 were most frequently recognized by IgE  
254 (7.6 – 2.4% patients), followed by Gal d 1 (ovomucoid, egg) and Bos d 4 (lactalbumin,  
255 milk) with 0.6% and 0.3%. These patterns were consistent in the population who did not  
256 receive AIT, although the prevalence of IgE recognition was lower as compared to the  
257 whole study population (Table 2).

### 258 ***Similarities and discrepancies between IgE and IgG recognition***

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

263 recognized by IgE antibodies ( $\leq 6\%$  subjects), were also recognized at low frequency (i.e.,  
264  $< 40\%$ ) by IgG (Phl p 7, Phl p 11, Bet v 4, Ole e 7, Amb a 1). On the other hand, other  
265 respiratory allergens also less frequently recognized by IgE antibodies were recognized in  
266  $> 80\%$  by IgG (Phl p 12, Bet v 2, Ole e 9). Similar patterns were observed in the population  
267 without AIT.

### 268 ***Geographical variation of IgE and IgG recognition***

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

273 For several major respiratory allergens the IgE and IgG frequencies in the different  
274 regions followed to a large extent the exposure levels with the corresponding allergen  
275 sources (e.g., ragweed exposure: Amb a 1; grass pollen exposure: Phl p 1 and exposure to  
276 cypress: Cup a 1) (Fig. 1 and table 3). For example, in agreement with similar levels of  
277 grass pollen exposure across France, IgE recognition frequencies for the major timothy  
278 grass pollen allergen, Phl p 1, were similar across the geographical areas (the adjusted-  
279 ORs for each center as compared to Paris varied between 0.68 to 1.29,  $p > 0.50$ ; Table 3)  
280 and IgG recognition of Phl p 1 was higher than 90% in each area. Inversely, IgE and IgG  
281 recognition frequencies for the cypress pollen allergen Cup a 1 showed marked  
282 geographical differences with adjusted OR of 7.4 (95%CI 2.1, 25.3) and 7.8 (95% CI 0.98-  
283 62.2) in Montpellier, the city showing the highest level of pollen exposures, as compared  
284 to Paris. Similar patterns were observed in subjects with and in subjects without asthma  
285 (Table E3), as well as in subjects without AIT (Table E4), although results should be  
286 interpreted cautiously because of the sample size .

287 However, for Bet v 1 the geographic pattern of IgE and IgG recognition differed. IgE  
288 recognition frequencies to Bet v 1 followed the exposure levels of birch pollen in the  
289 different regions whereas IgG recognition was high ( $> 60\%$ ) in all regions. This was also  
290 observed for food allergens that showed immunological cross-reactivity with Bet v 1 (PR-  
291 10 proteins, e.g., Mal d 1 from apple, Cor a 1.0401 from hazelnut, Ara h 8 from peanut,  
292 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

## 309 **Discussion**

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

## 328 **Strengths and weaknesses**

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

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

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

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



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

380

### 381 **Interpretation of the results**

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

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

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

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

422

423

424 **Acknowledgments**

425

426 The authors thanks the EGEA cooperative group:

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

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

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

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

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

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

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

453 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, Savvastianos 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



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

571

	n (%) / m (sd)
Number of males (%)	159 (46.8 %)
Mean age ( $\pm$ sd)	43.4 ( $\pm$ 16.8)
<b>Status at recruitment</b>	
<b>Asthma cases, n (%)</b>	83 (24.4)
<b>First-degree relatives of cases, n (%)</b>	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 <sup>3</sup> , n (%)	86 (25.4 %)
Atopic dermatitis ever, n (%)	127 (37.6 %)
FEV <sub>1</sub> < 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**

Allergen-source	Allergen	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	
		n	%	n	%	n	%	n	%
<b>Inhaled allergens</b>									
<b>Birch</b>	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
<b>Olive</b>	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
<b>Cypress</b>	nCup a 1	62	18.2	255	75.4	32	12.4	193	75.1
<b>Timothy Grass</b>	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
<b>Ragweed</b>	nAmb a 1	13	3.8	109	32.2	8	3.1	81	31.5
<b>House dust mite</b>	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
<b>Cat</b>	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
<b>Dog</b>	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
<b>Food allergens</b>									
<b>Apple</b>	rMal d 1	26	7.6	284	84.0	14	5.4	216	84.0
<b>Hazelnut</b>	rCor a	24	7.1						
	1.0401			234	69.2	12	4.7	176	68.5
<b>Peanut</b>	rAra h 8	16	4.7	233	68.9	6	2.3	180	70.0

<b>Peach</b>	rPru p 1	20	5.9	242	71.6	10	3.9	180	70.0
<b>Soy</b>	rGly m 4	8	2.4	292	86.4	2	0.8	221	86.0
<b>Milk</b>	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
<b>Egg</b>	nGal d 1	2	0.6	289	85.5			217	84.4
<b>Codfish</b>	rGad c 1	0	0.0	95	28.1			72	28.0

576

\* Allergen Immunotherapy

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	-				<b>3.56</b>	<b>1.81</b>	<b>7.02</b>	<b>&lt;0.01</b>	<b>0.001</b>	
	Marseille	-				<b>2.52</b>	<b>1.09</b>	<b>5.85</b>	<b>0.03</b>		
	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	<b>0.0009</b>	1.38	0.66	2.87	0.39	0.29
	Marseille	<b>3.56</b>	<b>1.17</b>	<b>10.9</b>	<b>0.03</b>		1.7	0.64	4.49	0.29	
	Montpellier	<b>7.37</b>	<b>2.15</b>	<b>25.3</b>	<b>&lt;0.01</b>		<b>7.79</b>	<b>0.98</b>	<b>62.2</b>	<b>0.05</b>	
	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	<b>0.37</b>	<b>0.15</b>	<b>0.96</b>	<b>0.04</b>	<b>0.01</b>	<b>13.6</b>	<b>1.68</b>	<b>110</b>	<b>0.01</b>	-
	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		<b>4.22</b>	<b>1.28</b>	<b>13.9</b>	<b>0.02</b>	
Der p 2	Lyon	<b>0.32</b>	<b>0.13</b>	<b>0.81</b>	<b>0.02</b>	<b>0.004</b>	<b>2.48</b>	<b>1.05</b>	<b>5.85</b>	<b>0.04</b>	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<sup>a,b,c</sup>, Christian Lupinek<sup>d</sup>, Yvonne Resch<sup>d</sup>, Mirela Curin<sup>d</sup>, Jocelyne Just<sup>e,f</sup>,  
Thomas Keil<sup>g,h</sup>, Renata Kiss<sup>d</sup>, Karin Lodrup Carlsen<sup>i,j</sup>, Erik Melén<sup>k,l</sup>, Rachel Nadif<sup>m,n</sup>,  
Isabelle Pin<sup>a,b,c</sup>, Ingebjørg Skrindo<sup>j,o</sup>, Susanne Vrtala<sup>d,p</sup>, Magnus Wickman<sup>l,m</sup>, Josep Maria  
Anto<sup>q,r,s,t</sup>, Rudolf Valenta<sup>d</sup>, Jean Bousquet<sup>m,n,u</sup>

**TABLE E1. 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 <sup>1</sup> , %	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 ( $\geq$ 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

<sup>1</sup> 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 $\geq 0.3$ 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	-				<b>2.60</b>	<b>1.20</b>	<b>5.65</b>	<b>0.02</b>
	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	<b>4.64</b>	<b>0.99</b>	<b>21.70</b>	<b>0.05</b>	1.71	0.55	5.36	0.36
	Montpellier	<b>13.79</b>	<b>2.64</b>	<b>72.02</b>	<b>0.00</b>	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	<b>3.39</b>	<b>1.00</b>	<b>11.47</b>	<b>0.05</b>
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)