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

3

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44

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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 ¹ whereas allergen-specific IgG antibodies occur in both
128 allergic and non-allergic individuals.² Allergen-specific IgG antibodies play a role in
129 allergen-specific clinical tolerance, either occurring naturally or induced by allergen-
130 specific immunotherapy.³⁻⁷ For certain allergens IgE and IgG responses differ.⁸ Allergen-
131 specific IgG may be associated with exposure to the respective allergen.^{9,10} 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.^{8,11} 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.¹²⁻¹⁶
144 The resulting MeDALL chip has been validated ^{15,16} and more than 6000 sera from 7
145 European birth cohorts (e.g., BAMSE and ECA)^{17,18} have been tested so far.

146 The Epidemiological study of the Genetics and Environment of Asthma, bronchial
147 hyperresponsiveness and atopy - EGEA ¹⁹⁻²¹ 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,¹⁵ 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.^{22,23} 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.²¹ 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.²³ 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 ≥ 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.^{15,16} 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.^{16,18,24}

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.¹⁸ 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>).^{25,26} Data are presented as the daily mean of grains/m³ 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 ≥ 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, respectively¹⁶⁻¹⁸. Ordinal categorical variables were also
224 studied both for IgE (<0.3 <1 <3 <10 <30 and ≥ 30 ISU) and IgG (<0.5 <1.5 <5 <15 <50 and
225 ≥50 ISU) because of the non-Gaussian distribution. Chi² 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 ≥ 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.^{27,28} 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)²⁹, 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 ⁸.
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 ³⁰. 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.^{15,16} 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.^{15,16} Furthermore, the allergen repertoire
376 on the MeDALL chip covers most of the relevant allergen sources.¹⁷ 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.¹⁷ 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.³¹ 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).³²⁻³⁴ Patients with a subclinical sensitization to birch pollen exist
392 in an area without atmospheric birch pollen exposure.³⁵ 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.²⁹ 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.^{36,37} 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)³⁸ and PR-10 proteins³⁹
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.¹¹ 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 skin⁴⁰ 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.¹⁸ The results of the present study will be valuable for better understanding
420 when allergen-specific interventions like avoidance or immunotherapy should be
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422

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569

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

571

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

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	%
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
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

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	-				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

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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 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 (\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

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)