Received: 12 September 2022 | Revised: 4 January 2023 | Accepted: 16 January 2023

DOI: 10.1111/all.15689

Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI WILEY

ORIGINAL ARTICLE

Epidemiology and Genetics

A molecular sensitization map of European children reveals exposome- and climate-dependent sensitization profiles

M. B. Gea Kiewiet[1] | Christian Lupinek[2] | Susanne Vrtala[2] | Sandra Wieser[2] | Alexandra Baar[2] | Renata Kiss[2] | Inger Kull[3,4] | Erik Melén[3,4,5] | Magnus Wickman[5] | Daniela Porta[6] | Davide Gori[7] | Ulrike Gehring[8] | Rob Aalberse[9] | Jordi Sunyer[10] | Marie Standl[11,12] | Joachim Heinrich[11,12] | Dagmar Waiblinger[13] | John Wright[13] | Josep M. Antó[14] | Jean Bousquet[15,16,17] | Marianne van Hage[1] | Rudolf Valenta[2,18,19,20]

[1]Division of Immunology and Allergy, Department of Medicine Solna, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden

[2]Division of Immunopathology, Department of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria

[3]Department of Clinical Science and Education Södersjukhuset, Karolinska Institutet, Stockholm, Sweden

[4]Sachs' Children's Hospital, Stockholm, Sweden

[5]Department of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

[6]Department of Epidemiology, Lazio Regional Health Service, ASL Roma 1, Rome, Italy

[7]Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy

[8]Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands

[9]Division of Research, Department of Immunopathology, Sanquin Blood Supply, Amsterdam, The Netherlands

[10]Insituto de Salud Global Barcelona, Barcelona, Spain

[11]Institute and Clinic for Occupational, Social and Environmental Medicine, University Hospital, LMU Munich, Germany

[12]Comprehensive Pneumology Center Munich, German Center for Lung Research, Munich, Germany

[13]Bradford Institute for Health Research, Bradford, UK

[14]Departament de Ciències Experimentals i de la Salut, Centre for Research in Environmental Epidemiology (CREAL), Hospital del Mar Research Institute (IMIM), CIBER Epidemiología y Salud Pública (CIBERESP), Universitat Pompeu Fabra, Barcelona, Spain

[15]ARIA, Montpellier, France

[16]Fraunhofer Institute for Translational Medicine and Pharmacology ITMP Allergology and Immunology, Berlin, Germany

[17]Corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Institute of Allergology, Charite-Universitätsmedizin Berlin, Berlin, Germany

[18]Laboratory of Immunopathology, Department of Clinical Immunology and Allergy, Sechenov First Moscow State Medical University, Moscow, Russian Federation

[19]National Research Center – Institute of Immunology FMBA of Russia, Moscow, Russian Federation

[20]Karl Landsteiner University for Healthcare Sciences, Krems, Austria

Abbreviations: IgE, immunoglobulin E; ISAC, multiplex specific IgE test.

M. B. Gea Kiewiet and Christian Lupinek shared first authorship.

Marianne van Hage and Rudolf Valenta were co-corresponding authors and shared last authorship.

Correspondence
Rudolf Valenta, Division of Immunopathology, Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Waehringer Guertel 18-20, 1090 Vienna, Austria
Email: rudolf.valenta@meduniwien.ac.at

Funding information
European Commission

Abstract

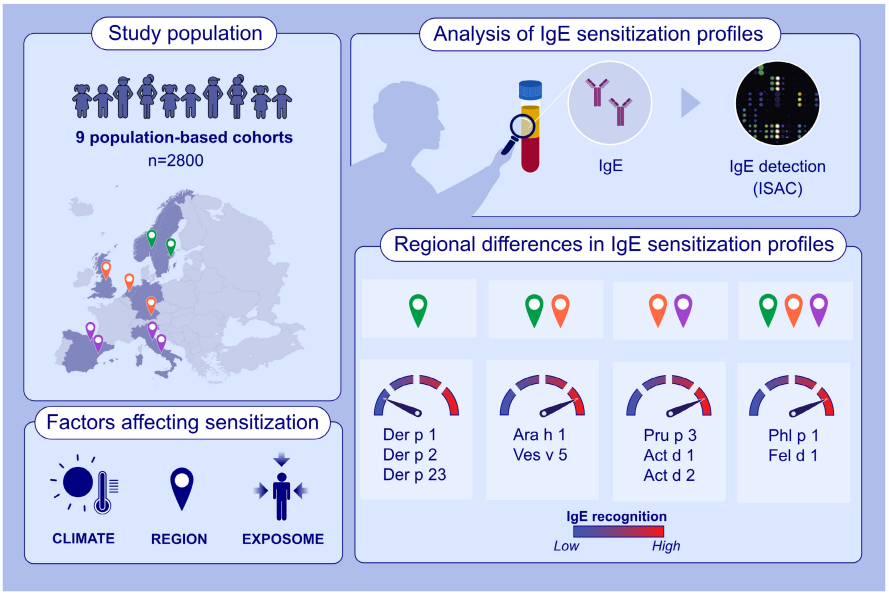
Background: Understanding differences in sensitization profiles at the molecular allergen level is important for diagnosis, personalized treatment and prevention strategies in allergy.

Methods: Immunoglobulin E (IgE) sensitization profiles were determined in more than 2800 sera from children in nine population-based cohorts in different geographical regions of Europe; north [BAMSE (Sweden), ECA (Norway)], west/central [PIAMA (the Netherlands), BiB (the United Kingdom), GINIplus (Germany)], and south [INMA Sabadell and Gipuzkoa (Spain) and ROBBIC Rome and Bologna (Italy)] using the MeDALL-allergen chip.

Results: Sensitization to grass pollen allergen, Phl p 1, and to major cat allergen, Fel d 1, dominated in most European regions whereas sensitization to house dust mite allergens Der p 1, 2 and 23 varied considerably between regions and were lowest in the north. Less than half of children from Sabadell which has a hot and dry climate were sensitized to respiratory allergens, in particular house dust mite allergens as compared to Gipuzkoa nearby with a more humid climate. Peanut allergen Ara h 1 was the most frequently recognized class 1 food allergen in Northern/Western Europe, while the fruit allergens Pru p 3, Act d 1 and 2 were prominent in Southern and Western/Central Europe. Ves v 5-sensitization dominated in North and West/Central Europe.

Conclusion: We show regional, exposome- and climate-dependent differences in molecular IgE-reactivity profiles in Northern, Western/Central and Southern Europe which may form a molecular basis for precision medicine-based approaches for treatment and prevention of allergy.

KEYWORDS
allergen molecules, Europe, exposome, IgE reactivity, MeDALL chip, sensitization profile



GRAPHICAL ABSTRACT

Sera from nine population-based birth cohorts representing Northern, West/Central, and Southern Europe were analyzed for IgE to more than 170 micro-arrayed allergen molecules. The sensitization map revealed exposome- and climate-dependent sensitization profiles, with strong regional differences. The results form a molecular basis for precision medicine-based approaches for prevention and treatment of allergy.
Abbreviations: IgE, immunoglobulin E; ISAC, multiplex specific IgE test

1 | INTRODUCTION

The prevalence of allergic diseases was increasing worldwide.[1–3] One may expect that allergic sensitization profiles differ between regions in Europe, due to variations in life style, genetics and the 'exposome', defined as the total exposure of the human body to environmental factors, in particular individual allergen molecules.[4] Understanding the sensitization patterns and their evolution over time in different regions is important for accurate diagnosis and will form the basis for novel treatment and prevention strategies across Europe.

In 2010, the European Union-funded project "MeDALL" (Mechanisms of the development of allergies) was initiated, a framework for research institutions specialized on various "omics"-technologies to join forces with groups conducting birth cohorts (https://cordis.europa.eu/project/rcn/96850/factsheet/en). This gave us the unique opportunity to compare the molecular IgE sensitization profiles from 9 different population-based cohorts located in different geographical regions of Europe; Northern [BAMSE[5] (Sweden), ECA[6] (Norway)], West/Central [PIAMA[7] (the Netherlands), BiB[8] (the United Kingdom), GINIplus[9] (Germany)], and Southern [INMA[10] Sabadell and Giupuzcoa (Spain) and ROBBIC[11] Rome and Bologna (Italy)] Europe. Together these cohorts comprised sera from more than 2800 children between the age of 1 and 16 years, allowing to compare also to some extent the evolution of sensitizations from early childhood to adolescence in the different regions of Europe. For this comprehensive IgE testing, a customized allergen microarray, the MeDALL-chip, was developed that covered 176 allergens and proved superior regarding sensitivity and coverage of allergen molecules as compared to available diagnostic tests.[12,13] The results of our analysis provide for the first time a comprehensive, high-resolution atlas of IgE-sensitization rates and patterns from the general population from different regions of Northern, Western/Central and Southern Europe.

2 | MATERIALS AND METHODS

2.1 | Cohorts and design of the study

Immunoglobulin E measurements were performed retrospectively on sera from 2855 children, aged 1–16 years, from nine different birth cohorts representing the northern, west/central and southern part of Europe. Two cohorts from Northern Europe, BAMSE[5] (Sweden) and ECA[6] (Norway), 3 cohorts from Western/Central Europe, PIAMA[7] (the Netherlands), BiB[8] (the United Kingdom), and GINIplus[9] (Germany), as well as four cohorts from Southern Europe, INMA[10] (Spain, Guipuzcoa and Sabadell) and ROBBIC[11] (Italy, Bologna and Rome) were included and information regarding the cohorts can be found in references[5–11] (Figure 1). For individual cohorts, blood collection had been scheduled for different ages. This allowed us to some extent to also investigate IgE sensitization between children of

FIGURE 1 Regions covered by the analyzed MeDALL-birth cohorts. Names of the respective cohorts and red circles indicate the regions that were covered by the study populations.

1, 4, 7–12 and 15–16 years of age. The exact location, participant age and numbers of analyzed sera of each cohort are summarized in Table 1. Sera were randomly picked within each cohort taking into consideration that only sera from children who were born in the region and spent at least the first year of life there were analyzed. Furthermore, we aimed at a gender balance regarding the samples. In those cohorts where different time points were studied sera were taken from children for whom samples were available at each of the time points of sampling. For each of the cohorts ethics approval and written informed consent from the parents or legal guardians of the children was available for the analysis of allergen-specific IgE.[5–11] The analysis of pseudonymized serum samples was performed at the Department of Pathophysiology and Allergy Research, Medical University of Vienna, Austria in a centralized manner with permission of the Ethics committee of the Medical University of Vienna, EK1641/2014. Possible limitations of the study are mentioned in Section 4 (https://www.strobe-statement.org/).

2.2 | MeDALL-chips

The customized MeDALL-chips were obtained from Phadia Austria GmbH, Part of Thermo Fisher Scientific ImmunoDiagnostics, A-1220, Vienna, Austria. Allergen microarrays were prepared according to the ImmunoCAP ISAC technology with some slight modifications and had been compared with traditional forms of allergy diagnosis in earlier studies.[12,13] More detailed information can be found in the supplementary information about quality controls and subsequent measures (Tables S1 and S2).

TABLE 1 MeDALL-cohorts and numbers of samples analyzed with the MeDALL-chip.

Cohorts		Numbers of sera by age							Chip- versions used
				8 years (BAMSE)					
Name	Country/Region	1 year	4 years	7–9 years (ROBBIC)	10 years	12 years	15 years	16 years	
BAMSE	Sweden/Stockholm		790	793				790	V1, V1.1, V2
ECA	Norway/Oslo				266			269	V1
PIAMA	Netherlands/Northern, western and central areas	107	107			107			V2
BiB	UK/Bradford (West Yorkshire)		250						V2
GINI	Germany/Munich and Wesel						343		V3
ROBBIC/Rome	Italy/Rome			415					V2
ROBBIC/Bologna	Italy/Bologna			175					V2
INMA/Sabadell	Spain/Sabadell (Catalonia)		302						V2
INMA/Guipuzcoa	Spain/Guipuzcoa (Basque region)		207						V3

Note: Samples obtained at 7–12 years (purple boxes) or at 15–16 years (yellow boxes) were combined in age groups.

2.3 | Data analysis

All analyses were performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp.). First, allergen molecules were grouped according to their exposure route. We identified a group of respiratory allergens, food allergens and 'other' allergens, which induce sensitization via different routes, including insect and latex allergens. Cross-reactive Carbohydrate Determinant (CCD)-bearing allergen molecules were analyzed as a separate group. Please note that the terms CCD marker and MUXF3 (i.e., Ana c 2.0101) are used in a synonymous manner throughout the manuscript and differ from the term "CCD-bearing allergen" which designates a protein allergen containing protein-bound CCDs. For each cohort and age group, allergic sensitization rates (percentage of IgE-positive subjects) were calculated for each allergen. All allergen molecules were ranked based on the sensitization frequencies and listed by group (Tables S3–S6). The median (minimum–maximum) ISU levels were also provided. From these tables, the 10 highest ranked primary (i.e., non-cross-reactive) allergens were extracted for each cohort and age (Figures 2–5). For these allergen molecules, the percentage of subjects with IgE levels were grouped according to ISU class ranges (low ≥ 0.3–1 ISU, moderate = 1–15 ISU, high > 15 ISU).

3 | RESULTS

3.1 | Frequencies of detectable molecular IgE sensitization to respiratory allergens and class I food allergens vary in the cohorts and increase by age but without major qualitative alterations within the cohorts with follow-up samples

Sensitizations to respiratory allergen molecules at 4 years of age were lowest in the INMA Sabadell cohort and highest in the BiB cohort (Figure 2). Sensitization to house dust allergen molecules at 4 and 7–12 years were low in the Nordic birth cohorts BAMSE and ECA but frequent in the other birth cohorts (Figure 2). Regarding class I food allergen molecules peanut allergens were frequently recognized in the BAMSE and BiB cohort but not in the other cohorts (Figure 3). Percentages of allergic sensitization and allergen-specific IgE levels increased with age in a similar manner in those cohorts where follow-up samples were available. However, no major changes in the qualitative sensitization profiles (i.e., hierarchies of IgE sensitizations) were observed between different age groups (Figures 2–5).

3.2 | Grass pollen allergens are the major pollen allergens in almost all European regions

The top-10 primary respiratory allergen molecules ranked by sensitization rate are shown in Figure 2. Timothy grass allergens were prominent in all cohorts from the age of 4, except in INMA Sabadell. At the age of 7–12 years, children in all cohorts were sensitized to the allergens Phl

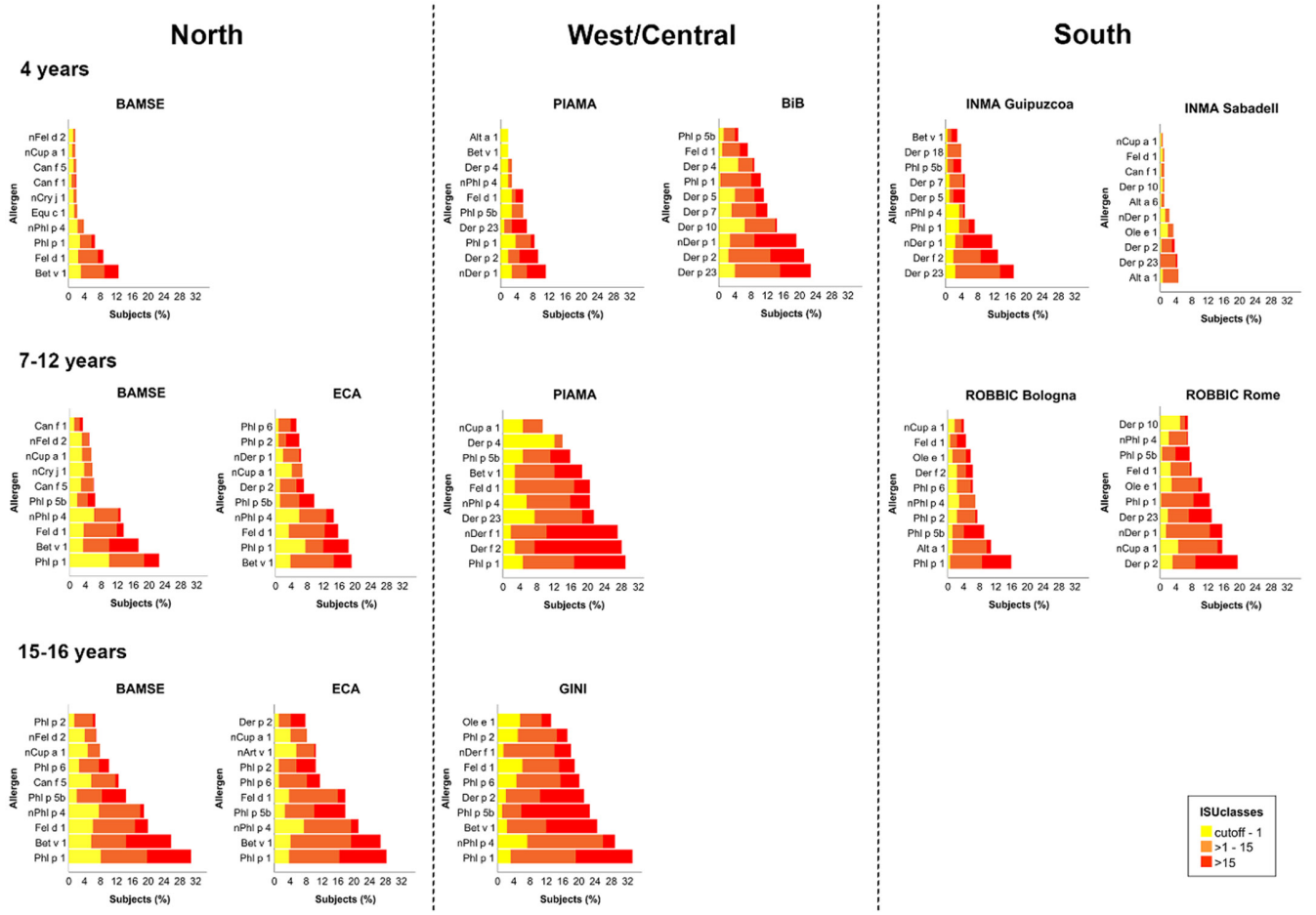


FIGURE 2 Overview of the 10 most frequently recognized primary respiratory allergens per cohort. The cohorts are organized based on age and region. For each cohort, allergens are ranked based on sensitization rate. Each bar shows the percentage of subjects with IgE levels within the different ISU classes (yellow ≥ 0.3–1 ISU, orange = 1–15 ISU, red > 15 ISU).

p 1, 5b, 6, 2, 11 and 12 (Table S3). Phl p 1 was the dominant allergen throughout all the cohorts. However, frequencies of IgE sensitization to Phl p 1 were highest in PIAMA, followed by BAMSE and ECA, and were lowest in the southern cohort ROBBIC. Phl p 7 was recognized in northern and western/central cohorts, but not in southern ones.

3.3 | Sensitization to tree and weed pollen allergens in the different European regions reflects the quality of allergen exposure, the exposome

The birch pollen allergen Bet v 1 was already an important allergen in the northern cohort BAMSE at a young age. As much as 12.5% of the 4 year olds had IgE reactivity against Bet v 1. In all other cohorts IgE recognition frequency of Bet v 1 was low. However, frequencies increased with age in all cohorts for which follow-up samples were available (i.e., ECA, PIAMA, BAMSE). At 12 years of age, Bet v 1 was also recognized by 19% of the children in the west/central cohort PIAMA, and at 15–16 years Bet v 1 was the second or third most recognized marker allergen in all cohorts from Northern, Central and Western Europe (around 25% in GINI, BAMSE and ECA; Figure 2).

In contrast, olive allergen Ole e 1-specific IgE was mainly detected in the southern cohorts. Both at the age of 4 and 7–12 years, Ole e 1 sensitization was higher in INMA and ROBBIC respectively, compared to all other cohorts. In addition, the cypress allergen Cup a 1 was prominent in the ROBBIC Rome cohort (Figures 2 and 5).

Regarding weed pollen allergens we found that the major mugwort allergen, Art v 1 was quite frequently recognized by children from the BAMSE and ECA cohort (Table S3 and Figure 2) and the major Parietaria allergen, Par j 2, showed frequent IgE reactivity in children from the ROBBIC cohort in Rome which fits to the vegetation profiles in these areas. Interestingly, the major ragweed allergen, Amb a 1, did not seem to be relevant in the cohorts tested by us.

3.4 | Fel d 1 is an important indoor allergen in almost all European regions whereas frequencies of sensitization to house dust mite allergens vary considerably

The cat allergen Fel d 1 was the most frequently recognized pet allergen molecules in all cohorts and ages except for INMA

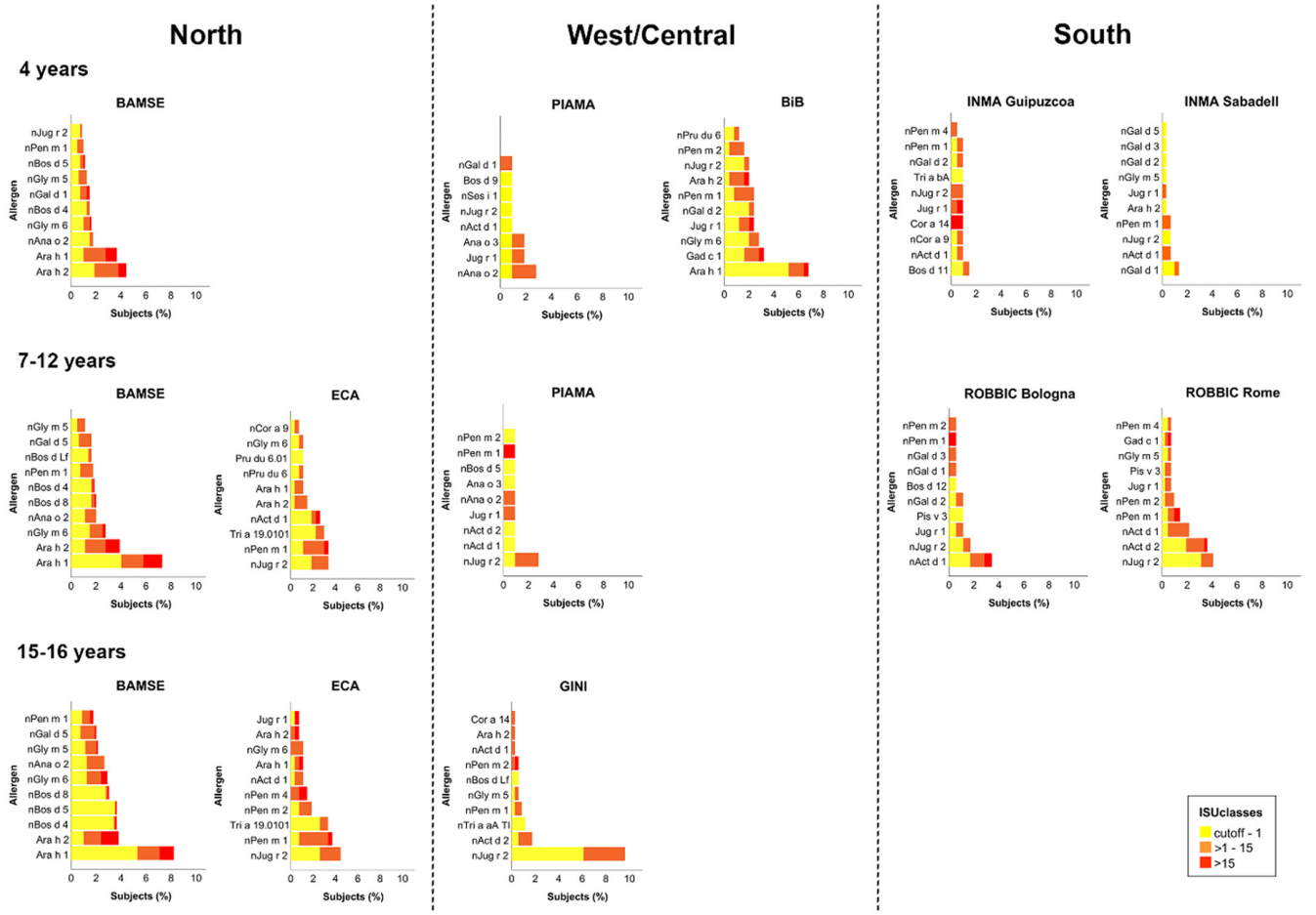


FIGURE 3 Overview of the 10 most frequently recognized type I food allergens per cohort. The cohorts are organized based on age and region. For each cohort, allergens are ranked based on sensitization rate. Each bar shows the percentage of subjects with IgE levels within the ISU classes (yellow ≥ 0.3–1 ISU, orange = 1–15 ISU, red > 15 ISU).

Guipuzcoa. At 4 years, the sensitization frequency to Fel d 1 was highest in BAMSE (8.7%), BiB (7.2%) and PIAMA (5.6%), whereas it was low in INMA Sabadell (1%) and INMA Guipuzcoa (0.5%; Figure 2). Around 20% of the oldest children (age 15–16 years) were sensitized to Fel d 1 in GINI, ECA and BAMSE (Figure 2 and Table S3). Sensitization to house dust mite allergens varied considerably in the different regions. For the western/central and southern cohorts, the house dust mite allergens Der p 1, 2 and interestingly also Der p 23 were among the allergen molecules with the highest recognition frequencies (Figure 2). Also, Der p 5, 7, 15, and 37 were often recognized (Table S3). However, house dust mite allergens were only minor allergens in the northern cohort BAMSE at all ages. We also noted striking differences regarding sensitization to house dust mites in Southern Europe. In Sabadell which is close to Guipuzcoa in Spain, less than half of the children were sensitized to house dust mite allergens (Figure 2). In addition, the fungus allergen Alt a 1 was prominent only in the southern cohorts. It was the most frequently recognized respiratory allergen in INMA Sabadell at 4 years and the second most recognized component in ROBBIC Bologna at 7–12 years (Figure 2).

3.5 | Genuine sensitization to peanut allergens is frequent only in certain regions

The top-10 class 1 food allergen molecules ranked by sensitization rate are shown in Figure 3. The major peanut allergen Ara h 1 was the most frequently recognized class 1 food allergen in the BiB cohort (6.8%) and the second most recognized in the BAMSE cohort (4.9%) at 4 years. In all other cohorts at this age the sensitization rate was very low. At older ages Ara h 1 was the most recognized allergen molecule in BAMSE, followed by Ara h 2. To a less extent it was also recognized in ECA, but not in western and southern cohorts. Other peanut components like Ara h 3, 6 and 9 were recognized in most cohorts, but in lower frequencies (Table S4).

In Southern Europe both at the ages of 4 and 7–12 years, as well as in Western/Central Europe, the kiwi allergens Act d 1 and 2 were among the most frequently recognized class 1 food allergen molecules, but not in BAMSE. However, the peach allergen Pru p 3 was the dominant class 1 food allergen in ROBBIC Rome, but not in ROBBIC Bologna at 7–12 years. Furthermore, the heat-stable and allergenic egg allergen Gal d 1 was most prominent in PIAMA at 1 year. Cow's milk allergens are represented in all but one cohort

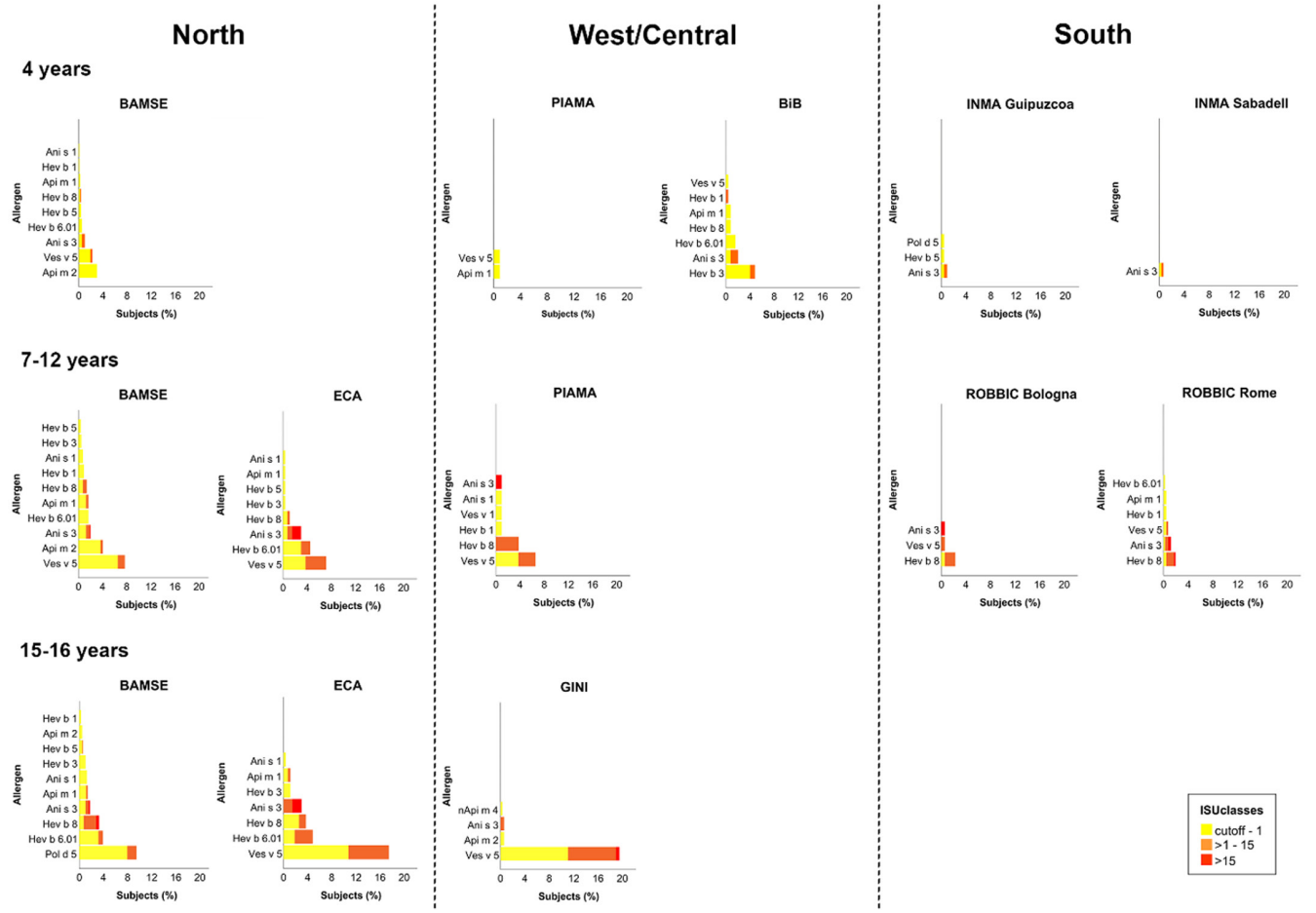


FIGURE 4 Overview of the 10 most frequently recognized primary other allergens per cohort. The cohorts are organized based on age and region. For each cohort, allergens are ranked based on sensitization rate. Each bar shows the percentage of subjects with IgE levels within the ISU classes (yellow ≥ 0.3–1 ISU, orange = 1–15 ISU, red > 15 ISU).

(INMA Sabadell) at all ages, but mostly in less than 1% of the children (Table S4). Besides class 1 food allergens, cross-reacting PR-10 proteins like Cor a 10,401, Mal d 1 and Pru p 1 are among the most frequently recognized molecules in cohorts with high Bet v 1 sensitization rates, due to cross-reactivity (Table S4).

3.6 | Wasp allergen Ves v 5 and other insect allergens are dominant allergen molecules in Northern and Western/Central Europe at all ages, but not in Southern Europe

The top-10 of other primary allergen molecules ranked by sensitization rate are shown in Figure 4. Ves v 5 sensitization from a young age was most frequent in the northern cohorts. Ves v 5 was most prevalent in BAMSE at the age of 4 (2.3%). In 7–12 year-old children Ves v 5 sensitization was around 7% in BAMSE, ECA and PIAMA, but low in ROBBIC Bologna and ROBBIC Rome. This frequency remained stable at the age of 15–16 years in BAMSE, but increased in ECA (17.5%). In many cohorts the paper wasp allergen Pol d 5 was recognized as well due to cross-reactivity with Ves v 5 (Table S5).

At the age of 7–12, recognition of latex components (Hev b 1, 3, 5, 6.01, 8) was observed in all cohorts, although most frequencies were below 2%. The latex profilin, Hev b 8, was the most frequently recognized allergen in both ROBBIC cohorts (around 2%), in the same frequency as the cross-reacting grass pollen profilin Phl p 12. At the older age of 15–16 years, children from the northern cohort showed a sensitization rate of around 4% against Hev b 6.01, but this was not observed in GINI.

3.7 | Sensitization to CCD-bearing allergens is dominated by grass pollen nPhl p 4 in Northern, Central and Western Europe and by nCup a 1 in the south

The timothy allergen nPhl p 4 was the most frequently recognized CCD-bearing allergen in all cohorts and in all age groups, except in ROBBIC Rome and INMA Sabadell (Figure 5). The sensitization rate increased with age in a similar manner in these cohorts (4 years: 2.8–4%, 7–12 years: 12.9% - 20%, 15–16 years: 19–28.9%; Table S6). Furthermore, the tree-derived CCD-bearing allergen Cup

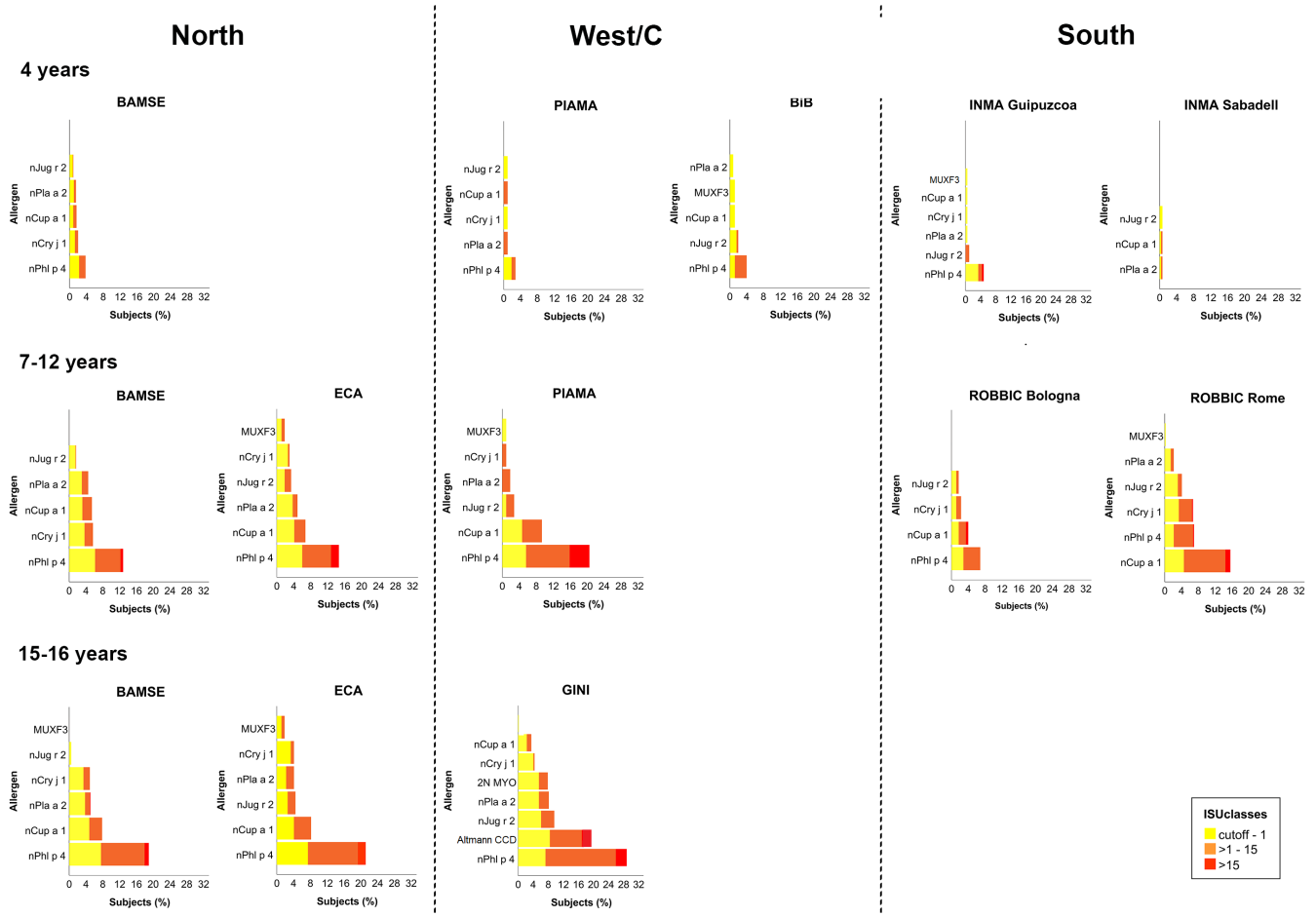


FIGURE 5 Overview of the most frequently recognized CCD-bearing allergens per cohort. The cohorts are organized based on age and region. For each cohort, allergens are ranked based on sensitization rate. Each bar shows the percentage of subjects with IgE levels within the ISU classes (yellow ≥ 0.3–1 ISU, orange = 1–15 ISU, red > 15 ISU).

a 1 (Cypress) was found to be prominent in the ROBBIC Rome cohort (15.7%) with approximately the double percentage compared to Phl p 4, while sensitization frequencies were low in all other cohorts. A frequently recognized CCD-containing food allergen was the walnut allergen Jug r 2. It was detected in all cohorts and age groups, mostly in relatively low frequencies (≤4.5%). Interestingly, sensitization rates to the pure CCD marker MUXF3 were similar in all cohorts and rather low (i.e., approximately 1–2%; Table S6).

4 | DISCUSSION

This study provides the first comprehensive overview of IgE-sensitization profiles at the molecular level in representative population-based cohorts of children and adolescents ($n > 100$ for each region) living in Northern, Western/Central and Southern Europe.

We found strong regional differences regarding IgE sensitizations to respiratory allergens (e.g., low IgE sensitization to house dust mite allergens in the Northern cohorts) which can be attributed to the climate in certain areas. Likewise, IgE sensitizations to class 1 food allergens varied which may depend on peculiarities of food consumption with some cohorts showing high IgE sensitization rates to genuine peanut allergen molecules (e.g., BAMSE, BiB) whereas peanut sensitization was lower in the other cohorts.

Striking regional differences regarding molecular IgE sensitization profiles in the different cohorts were observed. There are only few previous studies which have analyzed molecular IgE sensitization profiles in population-based cohorts from individual countries (e.g., the United Kingdom, Germany, Italy) which in fact confirm the molecular sensitization patterns which we observed for these countries.[14–16] However, there is only one study which involved different regions of France and demonstrated that there can be important differences regarding molecular sensitization profiles based on differences in the regional exposome. In fact, Siroux et al.[17] showed that the sensitization profile of people from five different regions even within on country (i.e., France) differed significantly, which was reflected in the differences in vegetation between the studied areas. Also in our study the exposome and in particular the climate seemed to show local differences as observed between the cities of Rome and Bologna as well as Sabadell and Gipuzkoa in Italy and in Spain, respectively.

The fact that the climate in Sabadell is much more hot and dry[18] than in Gipuzkoa[19] may be a reason why less than half of the children of the same age (i.e., 4 years) were sensitized to respiratory allergens, in particular to house dust mite allergens. Thus frequencies of IgE sensitization to respiratory allergens in children at 4 years were especially low in the dry and hot region of Sabadell as compared to cohorts from North-, West- and Middle Europe.

When scrutinizing differences between the cohorts, we first observed that Phl p 1, the major timothy grass pollen allergen, was the dominant allergen in all investigated regions due to the ubiquitous distribution of grasses. Phl p 1 has been suggested to initiate the sensitization process to timothy grass in pollen allergic children.[20,21] Furthermore, Phl p 1 is highly cross-reactive with group 1 allergens in different grass species and unlike other grass pollen allergen groups, group 1 allergens occur in all grass species,[22] which is reflected in the high frequency of sensitization against grasses in general in all regions. However, sensitization against Phl p 1 and other timothy grass allergens were not detected in subjects of the INMA Sabadell cohort. Again, this it likely due to the dry and hot climate there.[23]

For tree pollen allergens significant differences in sensitization profiles were found between Northern/Central and Southern Europe, which clearly reflect the different tree exposomes in these regions. Birch trees are most common in Northern and Central Europe.[24,25] In line with this, Bet v 1, the major birch allergen, was already prominent in the BAMSE and ECA cohorts (Northern Europe) at a young age, while it played a more significant role in PIAMA and GINI (Western/Central Europe) in older children suggesting an increase of detectable IgE sensitization by age. However, for most of the cohorts we did not have follow up samples to draw firm conclusions regarding the longitudinal development of IgE sensitizations and the associated development of symptoms. Such studies have been performed so far only for certain allergen sources and in certain cohorts[5,26–29] and were not the topic of our study which aimed to provide a comprehensive picture of molecular IgE sensitizations in different regions of Europe.

In contrast to Northern Europe, Italy and Spain, where olive trees are responsible for a significantly part of airborne pollens,[30] sensitization was observed in the INMA and ROBBIC cohorts. Cypress is another typical Mediterranean tree found above all in Italy[31] which was reflected in the dominance of Cup a 1 sensitization mainly in Rome. Like for Gipuzkoa and Sabadell, two close regions in Spain, we noted strong differences regarding molecular IgE sensitization profiles between Bologna and Rome. In Bologna sensitizations to grass pollen allergens dominated whereas in Rome sensitization to house dust mite allergens were more frequent. We think that it is an important finding of our study that we detected strongly varying molecular IgE sensitization profiles even in regions which are close to each other within one country because this finding has important implications for precision medicine approaches such as allergen avoidance (e.g., house dust mite allergy) and accurate prescription of allergen-specific immunotherapy. Molecular diagnosis is especially important for the precise identification of the genuinely sensitizing allergen sources which can be obscured by cross-reactivity when allergen extracts are used.

The major cat allergen Fel d 1 was the most frequently recognized allergen among the furry animals. When comparing the European regions, Fel d 1 sensitization was most common in Northern and Central Europe already from a young age, while sensitization frequencies were lower in Southern Europe. A similar profile was recently also described for the Moscow region of Russia, where Fel d 1 was the most frequently recognized indoor allergen.[32] The data is in line with a report showing that a higher percentage of people in Norway, Sweden and the United Kingdom had a cat during childhood.[33] However, since multiple factors have been found to affect Fel d 1 levels, including keeping cats indoors, smoking habits and ventilation, it still remains unclear why Fel d 1 levels in house dust are lower in southern Europe.[34] One possibility though may be that cats are less often kept indoor in these countries due to the climate.

The presence of house dust mite allergens, both Der p and Der f, depends on humidity.[35,36] This is in line with our finding that IgE to the house dust mite allergens were almost absent in BAMSE, very low in Sabadell, present in ECA and PIAMA and most prominent in the BiB cohort from the United Kingdom. In most cohorts sensitization was observed against several of the major house dust mite allergens, Der p 1, Der p 2, and Der p 23, as well as against other house dust mite allergens, like Der p 4, 5, 7 and 10.[37] Unlike house dust mites, the fungus *Alternaria alternata*, has shown to be an indoor allergen which grows better in a dry and warm climate. As a result, Alt a 1 was found to be one of the most important allergens only in the cohorts from Sabadell (Spain) and Bologna (Italy).

Regarding food allergens our study differs from the EuroPrevall study which has focused on food-allergic subjects and only few molecular analyses focusing on certain food allergens have been performed within EuroPrevall.[38] By contrast, our study has investigated random population samples from different parts of Europe for IgE sensitizations to food allergens. We found that Ara h 1 and 2 are clearly the most prominent allergens in BAMSE and BiB, but rare in the other cohorts. Geographical differences in clinical and immunological profiles of peanut allergens have been reported. Vereda et al. showed that peanut allergic patients from the US and Sweden recognized the storage proteins Ara h 1–3 more frequently compared to Spanish patients who were more often sensitized against the lipid transfer protein Ara h 9.[39] We also noted that Ara h 9 sensitization was higher in the southern cohorts INMA Gipuzkoa and ROBBIC Rome compared to BAMSE. These differences are not only depending on the amount and timing of peanut consumption. A study from Sweden has shown that the increase in peanut sensitization over the years is not only due to increased peanut consumption.[40] Differences in preparation of peanuts also plays a role. Roasted peanuts, which are consumed more in Sweden, the US and other western countries, contain more stable proteins and thus may have a higher allergenicity.[41] Regarding peanut differences of allergen contact via the skin may also be considered to be responsible for different sensitization rates in the different populations besides nutritional habits.[42] High sensitization rates to peanut allergens and to the major fish allergen Gad c 1 in the BiB cohort from the United Kingdom as compared to other cohorts may be an example for such nutritional habits.

However, sensitization against the dominant shrimp allergen Pen m 1 may reflect to some extent cross-reactivity with the tropomyosin Der p 10. In individual patients, specific IgE levels to Pen m 1 and Der p 10 and IgE cross-inhibition studies may inform which allergen may have been the genuinely sensitizing molecule. Act d 1 sensitization was found to be prominent only in southern Europe, where kiwifruit is grown locally, and especially Italy is known for its high kiwifruit consumption.[43]

Regarding venom allergens, our study provides new and unexpected information, since data on hymenoptera IgE sensitization are scarce, especially in children. We found that between 7 and 20% of 15–16 year olds from the northern cohorts showed IgE reactivity against the major wasp venom Ves v 5 while a considerably lower rate of sensitization was found at younger ages, which is in line with data reported previously.[44] Although we did not have data from Southern Europe for the 15–16 year olds, Ves v 5 sensitization seems to be less frequent in this area at a younger age. The most important wasp species, belonging to the Vespula genus and responsible for Ves v 5 sensitization, have been found to be present all over Europe, but more precise data on their geographical distribution and population density are lacking, which makes it difficult to explain the observed differences in sensitization frequency.[45] We speculate that children in Northern Europe could be more exposed to wasps, for example because they spend more time outdoors and in nature during the summer period.

With respect to IgE positivity to natural allergen molecules bearing cross-reactive carbohydrate determinants (CCDs), similar rates were observed throughout all regions of Europe, with Phl p 4 being the most prominent CCD-bearing allergen. For these CCD-bearing allergen molecules, coming mainly from plants, it is impossible to distinguish IgE reactivity to the sugar moieties from antibody binding to the protein backbone at an individual level. However, only in southern cohorts IgE levels to nCup a 1 were found to be indicative for true sensitization to those trees (cypress, cedar) or grasses (Bermuda grass) that are native in those regions. The remarkably high prevalence of IgE positivity to nJug r 2 in the German GINI-cohort can presumably be partly attributed to reactivity with CCDs present on this glycoprotein, while in other cohorts reactivity to nJug r 2 was paralleled by an increase of IgE to Jug r 1, indicative of genuine IgE sensitization to walnut. Regarding the only CCD marker (i.e., MUXF3) which was tested in each of the cohorts a relatively low frequency (approximately 1–2%) of IgE reactivity was found indicating that for CCD-bearing allergens also protein IgE epitopes play a role.

It is one limitation of our study that not all children from whom sera had been collected had exactly the same age but this should not affect the major findings of the study which are that sensitization profiles to allergen molecules seemed to vary regarding the allergen exposome and climate in the different cohorts and remained largely unaltered over time. Another limitation of our study is that we have not taken into account the atopic background of the parents of children when picking the serum samples from children but it seems that the atopic background of parents does not have such strong effects on allergic sensitization in children.[46] Likewise, we have not stratified children according to genetic background, ethnicity, nutritional habits and environmental pollution. However, in a recent study we did not find much evidence that pollution would influence allergen-specific IgE sensitization.[47] Other limitations of our study are that we make only descriptive comparisons without any adjustments and that the analyses were done only for available samples for arbitrarily selected cohorts. On the other hand one may consider the arbitrary analysis of children who were born and grew up in a region as a strength because it may provide real-life pictures of the local molecular sensitization profiles. Furthermore, to the best of our knowledge, our study revealing molecular sensitization profiles in a population-based cohorts of children from a continent represents the first of its kind in the world. A more detailed molecular IgE sensitization map of Europe and other continents may be obtained in the future by cross-sectional analyses of random populations of patients who are recruited by questionnaires from several different regions of the individual countries with different climate and living habits. Like in our study the patients should have been born and grown up in the regions of investigation to inform about the influence of the exposome and climate conditions on allergic sensitization.

In conclusion, this comprehensive data-set of high-resolution IgE-sensitization patterns of several thousand children from population-based European birth cohorts, with a north, south and west/central gradient, provides a detailed overview of regional differences in IgE-reactivity profiles of the general populations, which depend largely on the local exposome and climate. Since the method used for IgE detection was based on a commercially available platform (ImmunoCAP ISAC), our data can be combined with existing and future data-sets from further cohorts based on this technology. Furthermore, our sensitization map of Europe may form a basis for molecular strategies for prevention and therapy of allergy.

AUTHOR CONTRIBUTIONS

CL, JA, JB, and RV designed the study. CL and RK performed the experiments. CL and GK analyzed and interpreted the data. IK, EM, MW, K-HC, KL-C, DP, DG, HAS, RB, UG, MS, JH, DW, JW, SV, SW, and AB collected patients' material and/or prepared and characterized allergen molecules. CL, GK, MvH, and RV contributed to data interpretation and wrote the first draft of the manuscript. All authors critically reviewed the manuscript and approved the submitted version.

ACKNOWLEDGEMENTS

We thank Daniel Ebner, Thomas Schlederer, and Christian Harwanegg for excellent technical assistance regarding manufacturing of the customized allergen arrays which were made at Phadia Austria GmbH, Part of Thermo Fisher Scientific ImmunoDiagnostics, A-1220, Vienna, Austria. This paper is dedicated to Prof. Jean Bousquet for his amazing leadership in the MeDALL project.

FUNDING INFORMATION

The study was supported by the European FP7-program MeDALL, the Danube Allergy Research Cluster by the Country of Lower Austria, the Swedish Research Council, The Region Stockholm (ALF project), The Swedish Asthma and Allergy Association's Research Foundation, The Swedish Cancer and Allergy Foundation, The King Gustaf V 80th Birthday Foundation, The Swedish Heart-Lung Foundation, The Hesselman Foundation, by the European Commission, by Mead Johnson, Evansville, Indiana, USA, by Nestle, Vevey, Vaud, Switzerland, by a Wellcome programme grant [WT223601/Z/21/Z: Age of Wonder, a UK Medical Research Council (MRC) and UK Economic and Social Science Research Council (ESRC) grant: MR/N02439/1 and by the special program (Programmi speciali-Art. 12 bis, comma 6 D.lgs.229/99 Sanitaria e della Vigilanza sugli Enti)] funded by the Italian Ministry of Health.

CONFLICT OF INTEREST STATEMENT

R.V. receives research grants from HVD Biotech, Vienna, Austria and Worg Pharmaceuticals, Hangzhou, China. He serves as consultant for Worg and Viravaxx AG, Vienna, Austria. MvH has received lecture fee from Thermo Fisher Scientific. GK has no conflict of interest to declare. CL and SW are currently employees of MacroArray Diagnostics GmbH, Vienna, Austria. JB reports personal fees from Cipla, Menarini, Mylan, Novartis, Purina, Sanofi-Aventis, Teva, Uriach. He is shareholder of KYomed Innov and MASK-air-SAS. The rest of the authors report no conflict of interest.

ORCID

Susanne Vrtala https://orcid.org/0000-0003-4250-8243
Inger Kull https://orcid.org/0000-0001-6096-3771
Daniela Porta https://orcid.org/0000-0002-3431-2729
Ulrike Gehring https://orcid.org/0000-0003-3612-5780
Rob Aalberse https://orcid.org/0000-0002-3902-6596
Marie Standl https://orcid.org/0000-0002-5345-2049
Joachim Heinrich https://orcid.org/0000-0002-9620-1629
Jean Bousquet https://orcid.org/0000-0002-4061-4766
Marianne van Hage https://orcid.org/0000-0003-3091-1596
Rudolf Valenta https://orcid.org/0000-0001-5944-3365

REFERENCES

1. Reynolds LA, Finlay BB. Early life factors that affect allergy development. *Nat Rev Immunol*. 2017;17:518-528.
2. Peters RL, Koplin JJ, Gurrin LC, et al. The prevalence of food allergy and other allergic diseases in early childhood in a population-based study: HealthNuts age 4-year follow-up. *J Allergy Clin Immunol*. 2017;140:145-153.e8.
3. Sterner T, Uldahl A, Svensson Å, et al. The southern Sweden adolescent allergy-cohort: prevalence of allergic diseases and cross-sectional associations with individual and social factors. *J Asthma*. 2019;56:227-235.
4. Blumenthal MN. Genetic, epigenetic, and environmental factors in asthma and allergy. *Ann Allergy Asthma Immunol*. 2012;108:69-73.
5. Wickman M, Lupinek C, Andersson N, et al. Detection of IgE reactivity to a handful of allergen molecules in early childhood predicts respiratory allergy in adolescence. *EBioMedicine*. 2017;26:91-99. doi:10.1016/j.ebiom.2017.11.009
6. Lødrup Carlsen KC. The environment and childhood asthma (ECA) study in Oslo: ECA-1 and ECA-2. *Pediatric Allergy and Immunology, Supplement*. Vol 13. Blackwell Munksgaard; 2002:29-31.
7. Koopman LP, Smit HA, Heijnen MLA, et al. Respiratory infections in infants: interaction of parental allergy, child care, and siblings - the PIAMA study. *Pediatrics*. 2001;108:943-948.
8. Wright J, Small N, Raynor P, et al. Cohort profile: the born in Bradford multi-ethnic family cohort study. *Int J Epidemiol*. 2013;42:978-991.
9. Heinrich J, Brüske I, Cramer C, et al. GINIplus and LISAplus - design and selected results of two German birth cohorts about natural course of atopic diseases and their determinants. *Allergol Select*. 2017;1:85-95.
10. Guxens M, Ballester F, Espada M, et al. Cohort profile: the INMA-INfancia y medio Ambiente-(environment and childhood) project. *Int J Epidemiol*. 2012;41:930-940.
11. Porta D, Fantini MP. Prospective cohort studies of newborns in Italy to evaluate the role of environmental and genetic characteristics on common childhood disorders. *Ital J Pediatr*. 2006;32:350-357.
12. Skrindo I, Lupinek C, Valenta R, et al. The use of the MeDALL-chip to assess IgE sensitization: a new diagnostic tool for allergic disease? *Pediatr Allergy Immunol*. 2015;26:239-246.
13. Lupinek C, Wollmann E, Baar A, et al. Advances in allergen-microarray technology for diagnosis and monitoring of allergy: the MeDALL allergen-chip. *Methods*. 2014;66:106-119.
14. Custovic A, Sonntag HJ, Buchan IE, Belgrave D, Simpson A, Prosperi MCF. Evolution pathways of IgE responses to grass and mite allergens throughout childhood. *J Allergy Clin Immunol*. 2015;136:1645-1652.e8. doi:10.1016/j.jaci.2015.03.041
15. Huang X, Tsilochristou O, Perna S, et al. Evolution of the IgE and IgG repertoire to a comprehensive array of allergen molecules in the first decade of life. *Allergy*. 2018;73:421-430. doi:10.1111/all.13269
16. Scala E, Alessandri C, Bernardi ML, et al. Cross-sectional survey on immunoglobulin E reactivity in 23,077 subjects using an allergenic molecule-based microarray detection system. *Clin Exp Allergy*. 2010;40:911-921. doi:10.1111/j.1365-2222.2010.03470.x
17. Siroux V, Lupinek C, Resch Y, et al. Specific IgE and IgG measured by the MeDALL allergen-chip depend on allergen and route of exposure: the EGEA study. *J Allergy Clin Immunol*. 2017;139:643-654.e6.
18. https://www.google.com/search?q=sabadell%2C+spain+and+weather+averages&client=firefox-b-d&ei=sYYcY42jGLeJxc8PyLub6Aw&ved=0ahUKEwjNv4GyoIr6AhW3RPEDHcjdBs0Q4dUDCA0&uact=5&oq=sabadell%2C+spain+and+weather+averages&gs_lcp=Cgdnd3Mtd2l6EAMyBwgAEB4QogQyBQgAEKIEMgUIABCiBDIHCAAQHhCiBDIFCAAQogQ6DQgAEEcQ1gQQsAMQyQM6CggAEEcQ1gQQsANKBAhBGABKBAhGGABQ1wtYsy5gyDVoAXABeACAAVuIAc4HkgECMTaYAQCgAQHIAQjAAQE&sclient=gws-wiz. Accessed March 04, 2023.
19. https://www.google.com/search?q=gipuzkoa%2C+spain+and+weather+averages&client=firefox-b-d&ei=iYQcY_rMFcKMxc8Pla-vAk&ved=0ahUKEwi6teOqnor6AhVCRvEDHZXXC58Q4dUDCA0&uact=5&oq=gipuzkoa%2C+spain+and+weather+averages&gs_lcp=Cgdnd3Mtd2l6EAMyBQgAEKIEOgoIABBHENYEELADOgcIABAeEKIESgQIQRgASgQIRhgAUPYPWNuLAWDqmAFoAnABeACAAV2IAaAJkgECMTeYAQCgAQHIAQLAAQE&sclient=gws-wiz. Accessed March 04, 2023.
20. Hatzler L, Panetta V, Lau S, et al. Molecular spreading and predictive value of preclinical IgE response to Phleum pratense in children with hay fever. *J Allergy Clin Immunol*. 2012;130:894-901.
21. Westman M, Åberg K, Apostolovic D, et al. Molecular spreading and predictive value of preclinical IgE response to Phleum pratense in children with hay fever. *J Allergy Clin Immunol*. 2020;145:1174-1181.e6. doi:10.1016/j.jaci.2020.01.006

22. Gangl K, Niederberger V, Valenta R. Multiple grass mixes as opposed to single grasses for allergen immunotherapy in allergic rhinitis. *Clin Exp Allergy*. 2013;43:1202-1216.
23. Dadvand P, Sunyer J, Basagaña X, et al. Surrounding greenness and pregnancy outcomes in four Spanish birth cohorts. *Environ Health Perspect*. 2012;120:1481-1487.
24. Biedermann T, Winther L, Till SJ, Panzner P, Knulst A, Valovirta E. Birch pollen allergy in Europe. *Allergy*. 2019;74:1237-1248.
25. Smith M, Jäger S, Berger U, et al. Geographic and temporal variations in pollen exposure across Europe. *Allergy*. 2014;69:913-923.
26. Westman M, Lupinek C, Bousquet J, et al. Early childhood IgE reactivity to pathogenesis-related class 10 proteins predicts allergic rhinitis in adolescence. *J Allergy Clin Immunol*. 2015;135:1199-206.e1-11. doi:10.1016/j.jaci.2014.10.042
27. Asarnoj A, Hamsten C, Wadén K, et al. Sensitization to cat and dog allergen molecules in childhood and prediction of symptoms of cat and dog allergy in adolescence: a BAMSE/MeDALL study. *J Allergy Clin Immunol*. 2016;137:813-21.e7. doi:10.1016/j.jaci.2015.09.052
28. Asarnoj A, Hamsten C, Lupinek C, et al. Prediction of peanut allergy in adolescence by early childhood storage protein-specific IgE signatures: the BAMSE population-based birth cohort. *J Allergy Clin Immunol*. 2017;140:587-590.e7. doi:10.1016/j.jaci.2016.12.973
29. Posa D, Perna S, Resch Y, et al. Evolution and predictive value of IgE responses toward a comprehensive panel of house dust mite allergens during the first 2 decades of life. *J Allergy Clin Immunol*. 2017;139:541-549.e8. doi:10.1016/j.jaci.2016.08.014
30. D'Amato G, Cecchi L, Bonini S, et al. Allergenic pollen and pollen allergy in Europe. *Allergy*. 2007;62:976-990.
31. Sposato B, Liccardi G, Russo M, et al. Cypress pollen: an unexpected major sensitizing agent in different regions of Italy. *J Investig Allergol Clin Immunol*. 2014;24:23-28.
32. Elisyutina O, Lupinek C, Fedenko E, et al. IgE-reactivity profiles to allergen molecules in Russian children with and without symptoms of allergy revealed by micro-array analysis. *Pediatr Allergy Immunol*. 2021;32:251-263.
33. Svanes C, Heinrich J, Jarvis D, et al. Pet-keeping in childhood and adult asthma and hay fever: European community respiratory health survey. *J Allergy Clin Immunol*. 2003;112:289-300.
34. Heinrich J, Bedada GB, Zock JP, et al. Cat allergen level: its determinants and relationship to specific IgE to cat across European centers. *J Allergy Clin Immunol*. 2006;118:674-681.
35. Arlian LG, Morgan MS, Neal JS. Dust mite allergens: ecology and distribution. *Curr Allergy Asthma Rep*. 2002;2:401-411.
36. Peat JK, Tovey E, Mellis CM, Leeder SR, Woolcock AJ. Importance of house dust mite and Alternaria allergens in childhood asthma: an epidemiological study in two climatic regions of Australia. *Clin Exp Allergy*. 1993;23:812-820.
37. Thomas WR. Hierarchy and molecular properties of house dust mite allergens. *Allergol Int*. 2015;64:304-311.
38. Kummeling I, Mills ENC, Clausen M, et al. The EuroPrevall surveys on the prevalence of food allergies in children and adults: background and study methodology. *Allergy*. 2009;64:1493-1497. doi:10.1111/j.1398-9995.2009.02046.x
39. Vereda A, Van Hage M, Ahlstedt S, et al. Peanut allergy: clinical and immunologic differences among patients from 3 different geographic regions. *J Allergy Clin Immunol*. 2011;127:603-607.
40. Van OJ, Ahlstedt S, Bengtsson U, Hulthén L, Borres MP. Specific IgE antibodies to peanut in western Sweden – has the occurrence of peanut allergy increased without an increase in consumption? *Allergy*. 2001;56:573-577.
41. Maleki SJ, Viquez O, Jacks T, et al. The major peanut allergen, Ara h 2, functions as a trypsin inhibitor, and roasting enhances this function. *J Allergy Clinical Immunol*. 2003;112:190-195.
42. Brough HA, Nadeau KC, Sindher SB, et al. Epicutaneous sensitization in the development of food allergy: what is the evidence and how can this be prevented? *Allergy*. 2020;75:2185-2205. doi:10.1111/all.14304
43. Mohd Wani S, Ahmad M, Masoodi F. A review of production and processing of kiwifruit. *J Food Process Technol*. 2017;8:699.
44. Stemeseder T, Klinglmayr E, Moser S, et al. Cross-sectional study on allergic sensitization of Austrian adolescents using molecule-based IgE profiling. *Allergy*. 2017;72:754-763.
45. Fernández J. Distribution of vespid species in Europe. *Curr Opin Allergy Clin Immunol*. 2004;4:319-324.
46. Lupinek C, Hochwallner H, Johansson C, et al. Maternal allergen-specific IgG might protect the child against allergic sensitization. *J Allergy Clin Immunol*. 2019;144:536-548. doi:10.1016/j.jaci.2018.11.051
47. Melén E, Standl M, Gehring U, et al. Air pollution and IgE sensitization in 4 European birth cohorts-the MeDALL project. *J Allergy Clin Immunol*. 2021;147:713-722. doi:10.1016/j.jaci.2020.08.030

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Kiewiet MBG, Lupinek C, Vrtala S, et al. A molecular sensitization map of European children reveals exposome- and climate-dependent sensitization profiles. *Allergy*. 2023;78:2007-2018. doi:10.1111/all.15689