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

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| Original Article Topics: | Basic and Translational Allergy Immunology |
| News and Views Topics: | |
| Keywords: | allergens and epitopes, personlized medicine, precision medicine, pediatrics, prevention, pollen |
| Abstract: | **Background:** Understanding differences in sensitization profiles at the molecular allergen level is important for diagnosis, personalised treatment and prevention strategies in allergy. **Methods:** IgE sensitization profiles were determined in more than 2800 sera from children in 9 population-based cohorts in different geographical regions of Europe; north (BAMSE (Sweden), ECA (Norway)), west/central (PIAMA (the Netherlands), BiB (UK), 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. |
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# Point-by-point response template

**Date:** October 9, 2022
**Manuscript Number:** ALL-2022-00932
**Title of Article:** A molecular sensitization map of European children followed from childhood to adolescence reveals exposome- and climate-dependent sensitization profiles
**Name of the Corresponding Author:** Rudolf Valenta
**Email Address of the Corresponding Author:** Rudolf.valenta@meduniwien.ac.at



Dear Dr. Luo Zhang,
Our results, which are based on more than 2800 sera from random population-derived birth cohorts from different European regions, show that the molecular IgE sensitization profiles may differ not only between Northern, Western/Central and Southern Europe, but may also vary strongly between regions which are geographically quite close, e.g.,in Spain and Italy but differ regarding climate and exposome. The study is novel and unique because no molecular survey of IgE sensitizations of this magnitude has ever been performed for a continent. Our results highlight the need for the precise identification of the disease-causing allergen source at the molecular allergen level when treatment, such as allergen-specific avoidance and allergen-specific immunotherapy, is required which can be obscured by cross-reactivity when tested only with allergen extracts.

**Major changes and additions to the revised manuscript (please list):**
1. Following the reviewers suggestion the title has been changed to “A molecular sensitization map of European children reveals exposome- and climate-dependent sensitization profiles”.
2. Figure E1 has been deleted.
3. A detailed description of how sera were randomly picked has been added. In addition the ethics statement was add4ed to the main manuscript and the centralized measurement with micro-arrays was explained.
4. Following the reviewers suggestions the novelty of the study has been better explained in the revised discussion and emphasis was put on the results from the molecular analysis.

We would like to thank you and the reviewers for the valuable comments which without doubt have helped us to improve our manuscript.

**Specific Responses:**

**Reviewer: 1**

COMMENTS FOR THE AUTHOR(S)

The manuscript describes a collaborative analysis of sera from seven allergy birth cohorts in Europe, a remarkable international effort. The questions asked are important and have not been described before. They provide evidence that IgE mediated sensitivity to a variety of allergens differs in populations living in different parts of Europe, some in predictable ways, others that are surprising. The authors have been drawn careful conclusions well within their data. I only have minor suggestions that might clarify the presentation.

**Reply:** We thank the reviewer for the excellent summary of our study and the kind comments.

Abstract keywords – spelling of "personalized" should be corrected

**Reply:** Corrected to "personalised". Line 77 of revised manuscript.

Ln 143- The fact that sera were analyzed in a single central laboratory is an important point to my mind, greatly strengthening the quality of the data. In addition to the detailed methods described in the online repository, I would suggest a sentence in the methods, such as: "Serum aliquots were sent to the Department of Pathophysiology and Allergy Research (Vienna, Austria) for establishing IgE-reactivity profiles by microarray."

**Reply:** We thank the reviewer for pointing this strength of our study out and gladly followed the suggestion. Lines 139-148 of the revised manuscript.

Ln 297 – Here and in later parts of the manuscript, the source description should be moved to the reference section and given an appropriate reference number in the text.

**Reply:** We thank the reviewer for this suggestion and implemented it. The source descriptions have obtained reference numbers and were listed in the references. See lines 326-327 of revised manuscript.

Ln 313 "most dominant" should be simply "dominant"

**Reply:** Corrected as suggested, see line 333 of revised manuscript.

Ln330 – how is "most prominent " related to "most dominant"

This should reconcile for clarity

**Reply:** We agree and corrected to "most frequently recognized". See line 365 of revised manuscript.

Ln335- indoor cat allergen ref 25 check

**Reply:** We thank the reviewer for the insightful comment and checked the reference. In fact, reference 25 says that cat allergen levels are lower in Spain than in middle Europe and thus seems suitable. The paper also says "that not having a cat in the home is associated with substantially lower Fel d 1 concentration, but does not protect against high Fel d 1 exposure in communities where cat ownership is common." However, the latter statement does not seem to contradict our statement that cat allergen levels are lower in southern countries.

Ln341 – since the manuscripts data described presence of IgE antibody in

sera, not home allergen levels, this sentence should read: "This is in

line with our finding that IgE to house dust mite allergens was almost

absent in sera from the BAMSE cohort, very low in sera from the Sadabel

cohort ……"

**Reply:** We thank the reviewer and corrected the sentence, see line 376-377 of the revised manuscript.

Ln387 -I do not understand the caveat that sera were not collected at the

same time. According to Table 1, and subsequent figures, adequate samples

were available to support the conclusion that there were regional

differences. Perhaps they mean that the start dates for the various

cohorts were spread over time, so that they could not compare seasonal differences that might relate to “wet or dry” years or temporal changes that might relate to climate change. The authors should be specific.

**Reply:** We thank the reviewer. In fact we meant that children from the different cohorts did not have exactly the same age (see Table 1, BAMSE: 8 years; ROBBIC: 7-9 years; ECA: 10 years; PIAMA: 12 years). We have corrected our statement to make this more clear, see lines 433-434 of revised manuscript.

**Reviewer: 2**

COMMENTS FOR THE AUTHOR(S)

From multiple birth cohorts across Europe the dominant sensitizations at each age have been defined. These sensitizations are discussed in the context of exposures due to climate and other factors.

**Reply:** We thank the reviewer for the concise summary.

A few minor comments for consideration

1) The reader may expect and desire a map of sensitization. Perhaps it may be possible to have a graphic (similar to the Fig 1 map showing the cohorts) but with the results. I appreciate there are many results and they differ by age. Perhaps the sensitizations at each age group (Fig 5) could be overlayed onto fig 1 or the areas exploded to make room.

**Reply:** We thank the reviewer for this excellent comment. In fact we would have wished to draw a relatively simple overview of the molecular IgE sensitization profiles according to regions as suggested by the reviewer. However, it turned out that we found considerable differences in molecular IgE sensitization profiles in two regions of Spain and Italy which are relatively close to each other (i.e., Rome and Bologna, Italy; Sabadell and Gipuzkoa, Spain). It thus turns out that differences in molecular IgE reactivity profiles can vary strongly even in the same country. We have revised our manuscript to make this point more clear and hope for the understanding of the reviewer that we could not find a way to present the complex data in the simplified way as requested by the reviewer.

2) the citation of the web link say be more readable as a citation with the

Internet address in the reference list at the end

**Reply:** We completely agree with the reviewer and provided reference numbers for the internet links. See lines 323-325 of the revised manuscript.

**Reviewer: 3**

COMMENTS FOR THE AUTHOR(S)

The purpose of characterizing patients based on molecular investigations is fundamental and indispensable for a correct diagnosis. However, some limitations appear in this study. It is not possible to compare the increase with age of the allergic sensitization in different population if the patients were not examined at the same time. This manuscript contains important information, I suggest to rewrite it.

**Reply:** We thank the reviewer for the comment and must agree. It is indeed not possible to compare the increase of IgE sensitizations between the different cohorts because sera were obtained at different time points and for certain cohorts at only one time point. Only for certain cohorts we could make statements about the age-dependent evolution of IgE sensitizations. We have revised the manuscript accordingly. See lines 1-3, 119, 125, 187-194, 212, 346-351, 459-460 of the revised manuscript.

Table 1 and figure 2. Not all children have been followed from childhood to adolescence. Therefore, the comparison between the various populations is uncertain. Considering this, the title of the manuscript should be modified.

**Reply:** Again we must agree with the reviewer and rewrote the title “A molecular sensitization map of European children reveals exposome- and climate-dependent sensitization profiles”.

Authors should explain how the sera were randomly picked. The sera from 9 larger population-based cohorts, located in different geographical regions of Europe, were previously studied for atopic disease. More information

should be provided. Authorizations to employ the sera should be provided by local ethics committees and caregivers.

**Reply:** We thank the reviewer for the valuable comments. 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 analysed. Furthermore, we aimed at a gender balance regarding the samples. In those cohorts where different time points were analysed sera were taken from children for whom samples were available at each of the time points studies. 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 (see references 5-12). The analysis of pseudonymised 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. This information was included in the main revised manuscript, lines 139-148.

Page 12 line 172- 175. It is not possible to compare the increase with age of the allergic sensitization in different population if they were not examined at the same time, e.g., PIAMA population vs BAMSE, ECA and GINI population were not examined when they were one year old.

**Reply:** We agree with the reviewer and limited our statement to those cohorts where follow up samples were available. See lines 1-3, 119, 125, 187-194, 212, 346-351, 459-460 of the revised manuscript.

Page 10. Authors should explain why sensitization to Cup a 1 in southern population should be due to CCD and not to the Cup a 1 pectate lyase. According to literature data, Cup a 1 is one of the main allergens in Rome (Italy) Pag 18. The sentence “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” should be better explained. The presence of the cross-reactive carbohydrate determinants (CCD) makes it difficult to draw firm conclusions on any association. Walnut contains more than one

allergen and Jug r 2 and Jug r 1 are different seed storage proteins. One patient might be sensitized to Jug r 1 and to Jug r 2 CCD but he could be also sensitized to the 7s vicilin Jug r 2. Rather than looking for an association with different molecules coming from walnut, it would be better to look on the chip for the sensitization to other 7s vicilin coming from different sources.

**Reply:** The reviewer makes again a very good comment. It is indeed not possible to use natural CCD-bearing allergens as CCD markers because IgE antibodies may be directed to their peptide epitopes and/or CCDs to a varying degree. We therefore focused on IgE positivity to the CCD marker MUXF3 and clarified in the manuscript that the terms MUXF3 and CCD marker are identical because only MUXF3 (Ana c 2.0101) was present on all chip versions (see Table E1). If one defines CCD-positivity according to MUXF3 sensitization the percentage of CCD-positive subjects is quite comparable among the cohorts. The frequency of IgE-positivity to CCD-bearing allergens may only serve as an indicator what CCD-bearing allergen may have been responsible for inducing CCD-specific IgE in addition to the peptide-specific IgE. We have carefully revised the manuscript with the goal to avoid the misunderstanding that the term “CCD-bearing allergen” may be confused with the term CCD marker (i.e., MUXF3). See lines 165-168 of revised manuscript. Following the reviewers suggestion to look for associations of IgE reactivities to 7s vicilins we were not successful to find such associations regarding the 7s vicilins Jug r 2, Ara h 1, Gly m 5, Pis v 3 and Ana o 1 which we had on the chip (see Figure 3). This may be or is probably due to the fact that the sequence homologies among these allergens are not very high.

The nomenclature of the table E6 should be made uniform with the name of the exact CCD marker present on the chip which instead is sometimes named “marker” and other times as MUXF3, other times it is missing (BAMSE, PIAMA, INMA, ROBBIC). Additionally, Authors should discuss the low percentage of sensitization towards MUXF3 compared to other CCD bearing proteins since this protein is considered one of the main markers of CCDs.

**Reply:** Following the reviewers suggestion we corrected Table E6 to make clear that MUXF3 was meant (see revised Table E6). As already indicated above we have revised the manuscript to address the discrepancies of IgE recognition frequencies to CCD-bearing allergens and the CCD marker MUXF3 see:" It is indeed not possible to use natural CCD-bearing allergens as CCD markers because IgE antibodies may be directed to their peptide epitopes and/or CCDs to a varying degree. We therefore focused on IgE positivity to the CCD marker MUXF3 and clarified in the manuscript that the terms MUXF3 and CCD marker are identical because only MUXF3 (Ana c 2.0101) was present on all chip versions (see Table E1). We have tested other CCD markers (i.e., 2N MYO, Altmann´s CCD blocker as indicated in Table E1) only in the GINI cohort. If one defines CCD-positivity according to MUXF3 sensitization the percentage of CCD-positive subjects is quite comparable among the cohorts. The frequency of IgE-positivity to CCD-bearing allergens may only serve as an indicator what CCD-bearing allergen may have been responsible for inducing CCD-specific IgE in addition to the peptide-specific IgE. We have carefully revised the manuscript with the goal to avoid the misunderstanding that the term "CCD-bearing allergen" may be confused with the term CCD marker (i.e., MUXF3)." Lines 422-432.

Figure E1. This figure might be misleading. It does not seem correct to state that this figure reports the sensitization rate per age group for each sensitization route. Not all children of the examined population were followed from childhood to adolescence.

**Reply:** We agree with the reviewer and deleted Figure E1 and the corresponding text as it is indeed misleading and built on incomplete data from different cohorts.

**Reviewer: 4**

COMMENTS FOR THE AUTHOR(S)

The current manuscript provides IgE sensitization data obtained from 2800 sera. The chip based study was performed within MeDALL. The serum samples were selected from different birth cohort studies (BAMSE, ECA, PIAMA, BiB, GINIplus, INMA and PIAMA and ROBBIC). This approach enables access to a large number of serum samples and allows comparing different exposure scenarios. The authors state: "These sera have been randomly picked, therefore representing the general population." This needs a more

detailed description since the different prospective cohort studies had applied different inclusion criteria and different primary goals ranging from identifying risk factors for allergic diseases, assessing onset of allergic manifestations, genetic predisposition up to assessing different nutritional habits, ethnicities, and environmental pollution. Therefore the samples might be heterogeneous and may affect the conclusions drawn from these findings.

**Reply:** We thank the reviewer for the valuable comments. Sera were randomly picked taking into consideration only that children were born in the region and spent at least the first year of life there. Furthermore, we aimed at a gender balance regarding the analysed sera. In the cohorts where different time points were analysed sera were taken from children for whom samples were available at all time points analysed. Our approach is thus indeed a quite random approach because it only takes gender balance and allergen exposure in the given area into account. However, we have discussed that factors such as atopic background of the parents could be a limitation of our study. Following the reviewers recommendation we included the fact that we did not make a selection according to genetic background of children, nutritional habits, ethnicities and environmental pollution, which, as we have shown earlier may have a modest, if any, effect (Air pollution and IgE sensitization in 4 European birth cohorts-the MeDALL project. Melén E, Standl M, Gehring U, Altug H, Antó JM, Berdel D, Bergström A, Bousquet J, Heinrich J, Koppelman GH, Kull I, Lupinek C, Markevych I, Schikowski T, Thiering E, Valenta R, van Hage M, von Berg A, Vonk JM, Wickman M, Wijga A, Gruzieva O. J Allergy Clin Immunol. 2021 Feb;147(2):713-722. doi: 10.1016/j.jaci.2020.08.030. Epub 2020 Sep 11) as additional limitations of our study (see lines 445-455 of the revised manuscript). On the other hand we think that the approach of analysing randomly picked samples has also strengths as it provides a real-life snapshot of the molecular IgE sensitization profiles in different regions and the samples sizes were not too small and surely provided reliable results regarding the molecular IgE sensitization profiles.

The chip based approach is sound and well done and supported by an already commercially available product. Another limitation of the current manuscript is the lack of discussion comparing the obtained data with data from other studies such as e.g. EUROPREVALL and others.

**Reply:** We thank the reviewer for pointing out the technical strength of our allergen microarray. We agree that it is appropriate to make comments regarding other studies. In fact the EuroPrevall study has first focused on food allergic subjects and not investigated random population samples especially not of children with the same age from a particular region. EuroPrevall has also not performed a comprehensive analysis of molecular IgE sensitizations against large panels of respiratory allergens, food allergens, other allergens such as venoms and carbohydrates at the same time (The EuroPrevall surveys on the prevalence of food allergies in children and adults: background and study methodology. Kummeling I, Mills ENC, Clausen M, Dubakiene R, Pérez CF, Fernández-Rivas M, Knulst AC, Kowalski ML, Lidholm J, Le TM, Metzler C, Mustakov T, Popov T, Potts J, Van Ree R, Sakellariou A, Töndury B, Tzannis K, Burney P. Allergy. 2009 Oct;64(10):1493-1497. doi: 10.1111/j.1398-9995.2009.02046.x. Epub 2009 Apr 6). We have mentioned this in our revised discussion (see lines 383-386). There are only a few other studies performed with smaller panels of allergen molecules which are limited to certain areas in Europe (e.g, UK: Evolution pathways of IgE responses to grass and mite allergens throughout childhood. Custovic A, Sonntag HJ, Buchan IE, Belgrave D, Simpson A, Prosperi MCF. J Allergy Clin Immunol. 2015 Dec;136(6):1645-1652.e8. doi: 10.1016/j.jaci.2015.03.041. Epub 2015 May 8; Germany: Evolution of the IgE and IgG repertoire to a comprehensive array of allergen molecules in the first decade of life. Huang X, Tsilochristou O, Perna S, Hofmaier S, Cappella A, Bauer CP, Hoffman U, Forster J, Zepp F, Schuster A, D'Amelio R, Wahn U, Keil T, Lau S, Matricardi PM. Allergy. 2018 Feb;73(2):421-430. doi: 10.1111/all.13269. Epub 2017 Oct 9 and Italy: Cross-sectional survey on immunoglobulin E reactivity in 23,077 subjects using an allergenic molecule-based microarray detection system. Scala E, Alessandri C, Bernardi ML, Ferrara R, Palazzo P, Pomponi D, Quaratino D, Rasi C, Zaffiro A, Zennaro D, Mari A. Clin Exp Allergy. 2010 Jun;40(6):911-21. doi: 10.1111/j.1365-2222.2010.03470.x. Epub 2010 Mar 1). We quoted these studies in the revised discussion and actually found that they support our data regarding the molecular IgE sensitization profiles in the UK, Italy and Germany (see lines 313-317 of revised manuscript)-

Moreover, the studies have already started up to 20 years ago and the data

should be available how the sensitization profiles led to allergic

diseases and the underlying sensitization profiles. Linking the current

data with the clinical datasets would improve the outcome of this study.

**Reply:** We thank the reviewer for this good comment but the goal of our study was to determine the molecular IgE sensitization profiles and not the longitudinal development of IgE sensitizations towards clinical symptoms later on. In fact, we and others have earlier performed several such analyses for cohorts with large numbers of longitudinally collected samples from the same children. However, this would have been outside the scope of our current study and difficult to perform because sample sizes from the individual cohorts analysed in this study

were lower. Nevertheless, we have quoted our longitudinal studies in the revised discussion (see lines 348-351).

Detailed criticism: It is obvious and expected that different geographical areas account for different aeroallergen sensitization patterns and this has already been published in several publications. “We show regional, exposome and climate dependent differences in molecular IgE-reactivity profiles ….which may form a ….basis for precision medicine-based approaches for treatment and preventions of allergy” This statement remains unclear and needs refinement. Regarding grass pollen allergens as the most prevalent pollen allergens across Europe is shown. It would be interesting to know if weed pollens in southern Europe are of that relevance in quantity and quality as they have been described in the literature.

**Reply:** Following the reviewers suggestion we have refined our statement. In fact, there are only a few studies which have analysed molecular sensitisation profiles but there is only one by Siroux which has looked into different regions of a country and described strong differences I molecular sensitization profiles (i.e., reference 14 in our paper: Specific IgE and IgG measured by the MeDALL allergen-chip depend on allergen and route of exposure: The EGEA study. Siroux V, Lupinek C, Resch Y, Curin M, Just J, Keil T, Kiss R, Lødrup Carlsen K, Melén E, Nadif R, Pin I, Skrindo I, Vrtala S, Wickman M, Anto JM, Valenta R, Bousquet J. J Allergy Clin Immunol. 2017 Feb;139(2):643-654.e6. doi: 10.1016/j.jaci.2016.05.023. Epub 2016 Jun 22). In fact, it was very interesting for us to see that there can be quite substantial differences between regions in Spain and Italy which are not too far from each other but differ regarding climate and exposome. We have refined our statement to reflect this finding better. Furthermore we named precision medicine-based forms of primary prevention and treatment such as allergen-specific avoidance and allergen-specific immunotherapy. In particular allergen-specific immunotherapy requires the precise identification of the disease-causing allergen source which can be obscured by cross-reactivity when only tested with allergen extracts. See lines 313-320, 355-364 of the revised manuscript.

The reviewer makes another very good point regarding the importance of weed pollen allergens. In fact, allergy to weed pollen allergens seems to be rather limited to certain specific

regions. For example, ragweed allergy is extremely common and frequent in the Lyon region in France but does not play an important role in the rest of the country (see reference 14 in our paper: Specific IgE and IgG measured by the MeDALL allergen-chip depend on allergen and route of exposure: The EGEA study. Siroux V, Lupinek C, Resch Y, Curin M, Just J, Keil T, Kiss R, Lødrup Carlsen K, Melén E, Nadif R, Pin I, Skrindo I, Vrtala S, Wickman M, Anto JM, Valenta R, Bousquet J. J Allergy Clin Immunol. 2017 Feb;139(2):643-654.e6. doi: 10.1016/j.jaci.2016.05.023. Epub 2016 Jun 22). We have now mentioned in the revised results IgE sensitization to certain weeds like mugwort and Parietaria as documented by Art v 1 and Par j 2 sensitization, respectively to highlight their relevance in different parts of Europe. See lines 220-224 of the revised manuscript.

Regarding class 1 food allergens peanut allergens are observed in certain areas – is there a good explanation why not equally distributed throughout Europe? Is this really a matter of food processing? What about different eating habits and life style? What about consumption rates of tree nuts such as hazelnut and walnut? Different sensitization patterns for HMD allergens are also provided in this study. While the north south difference for sensitization rate is known from other studies it is interesting to see that HMD sensitization is higher in southern dry climate as compared to a southern humid area. Is there an explanation for this finding?

**Reply:** We thank the reviewer for the good suggestions to highlight the topic of class 1 food allergens. Indeed we think that sensitisation to class 1 food allergens is related to eating habits and lifestyle but also other possibilities of sensitisation, for example via the skin in case of peanut may be considered. See revised manuscript lines 397-402.

Regarding IgE sensitization there seems to be a misunderstanding. We report that IgE sensitization to house dust mite allergens is more common in the warm and humid region of Gipuzkoa in Spain than in the warm and dry region of Sabadell in Spain. We have clarified this in the revised manuscript, see lines 326-329.

**Reviewer: 5**

COMMENTS FOR THE AUTHOR(S)

Gea Kiewiet et al sought to investigate the molecular IgE sensitization map of Europe by analysing 2800 sera from children of several cohorts from different regions of Europe (North, Central/Western and South) with micro-arrayed purified allergens. The authors show regional, exposome and climate-dependent differences in molecular IgE-reactivity profiles within the studied regions from Europe. Apart from the limitations of the study indicated in the discussion section, the authors should further explain and discuss the following issues:

1. What is the novelty of the obtained results and clinical translation/impact of the reported data/discussions? The authors should clearly state what are the new findings (not in terms of study design but in terms of data/conclusions) reported in this manuscript.

**Reply:** We thank the reviewer for allowing us to present the new finding better. In fact, as pointed out in our reply to reviewer 4 we would like to repeat"It was very interesting for us to see that there can be quite substantial differences in molecular IgE sensitization profiles and frequencies of IgE sensitization between regions which are geographically quite close, e.g.,in Spain and Italy but differ regarding climate and exposome. We have pointed this out in the revised discussion. Furthermore we named precision medicine-based forms of primary prevention and treatment such as allergen-specific avoidance and allergen-specific immunotherapy. In particular allergen-specific immunotherapy requires the precise identification of the disease-causing allergen source which can be obscured by cross-reactivity when only tested with allergen extracts. See lines 313-320, 355-364 of the revised manuscript."

2. The selected regions seem rather arbitrary and not necessarily resembling the heterogeneity reported in such European regions. The included areas do not fully represent such heterogeneity.

This should be discussed in more detail.

**Reply:** We agree with the reviewer. In fact, it is one limitation of our study that we have analysed only sera from regions where birth cohorts had been established. We think that it will be important to continue to study the investigation of molecular IgE sensitization profiles by conducting cross-sectional studies analysing sera from subjects who were born and grew up in different regions of different European countries to obtain a high resolution picture. This was mentioned in the revised discussion lines 445-449.

3. This is a retrospective cross-sectional study including sera from children aged 1-16 years. As this is not a prospective follow-up study, some of the reported conclusions in terms of onset and development of IgE sensitization profiles should be ameliorated.

**Reply:** Following the reviewers recommendation we ameliorated conclusion regarding onset and development of IgE sensitization profiles, see lines 1-3, 119, 125, 187-194, 212, 346-351, 459-460 of revised manuscript.

4. Is UK regiotype comparable to Central/Western Europe countries (i.e. Germany)?

**Reply:** We thank the reviewer for this good question. Despite the fact that sera obtained in the BiB cohort were from young children (age 4) and whereas German children were older (i.e., 15-16 years) one can say that the profile of recognized respiratory allergens was similar with the exception of house dust mite allergens which were more frequently recognized in the UK cohort. Regarding class 1 food allergens it seems that sensitization to peanut allergen molecules and to the major fish allergen Gad c 1 is highly frequent in the UK children but not in the children from Germany. We mentioned this interesting findings in the revised discussion (see lines 400-402 of revised manuscript).

5. The discussion is too long. It should be shortened to avoid redundancy of data already presented in the result section.

**Reply:** Following the reviewers suggestion we shortened the discussion by removing repetitions of results, see lines 300-313 of revised manuscript.

Sincerely yours,

Rudolf Valenta

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

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**Running title:** Molecular sensitization map of Europe

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**Abstract**

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

**Methods:** IgE sensitization profiles were determined in more than 2800 sera from children in 9 population-based cohorts in different geographical regions of Europe; north (BAMSE (Sweden), ECA (Norway)), west/central (PIAMA (the Netherlands), BiB (UK), 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***,*** IgE-reactivity, Europe, exposome, MeDALL chip, sensitization profile

## 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](UK), 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 to 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.

# Materials and methods

## Cohorts and design of the study

IgE 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] (UK), 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 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[5-12]. The analysis of pseudonymised 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 the discussion section. (https://www.strobe-statement.org/).

## 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 E1-E2).

## Data analysis

All analyses were performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA). 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 Determinants (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 designate protein allergens 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 E3-E6). 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).

# Results

## 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 four 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 years 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).

## 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 p 1, 5b, 6, 2, 11 and 12 (Table E3). 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.

## 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 (Figure 2, Figure 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 E3, 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.

**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 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, Table E3). 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 E3). However, house dust mite allergens were only minor allergens in the northern cohort BAMSE at all ages. However, 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).

**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 E4).

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 (INMA Sabadell) at all ages, but mostly in less than 1% of the children (Table E4). Besides class 1 food allergens, cross-reacting PR-10 proteins like Cor a 10401, 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 E4).

**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 E5).

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.

**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 E6). Furthermore, the tree-derived CCD-bearing allergen Cup 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 ~~recognized~~ 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 E6).

## 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., UK, Germany, Italy) which in fact confirm the molecular sensitization patterns which we observed for these countries[14, 15, 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 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.[17] 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 four 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[26-30] 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[31], sensitization was observed in the INMA and ROBBIC cohorts. Cypress is another typical Mediterranean tree found above all in Italy[32] 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 HDM 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., HDM 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.[33] The data is in line with a report showing that a higher percentage of people in Norway, Sweden and the UK had a cat during childhood.[34] 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.[35] 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.[36, 37] 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 UK. 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 HDM allergens, like Der p 4, 5, 7 and 10.[38] Unlike house dust mites, the fungus *Alternaria alternata*, has shown to be an indoor allergen which grows better in a dry and warm climate.[22] 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[39]. 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.[40] 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.[41] 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.[42] 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.[43] High sensitization rates to peanut allergens and to the major fish allergen Gad c 1 in the BiB cohort from UK 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.[44]

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.[45] 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.[46] 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[47]. 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[48]. 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.

**Authors contribution:**

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, K L-C, DP, DG, HAS, RB, UG, MS, JH, DW, JW, SV, SW, AB collected patients' material and/or prepared and characterized allergen molecules. CL, GK, MvH, RV contributed to data interpretation and wrote the first draft of the manuscript. All authors critically reviewed the manuscript and approved the submitted version.

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is shareholder of KYomed Innov and MASK-air-SAS. The rest of the authors report no conflict of interest.

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## Figures and legends

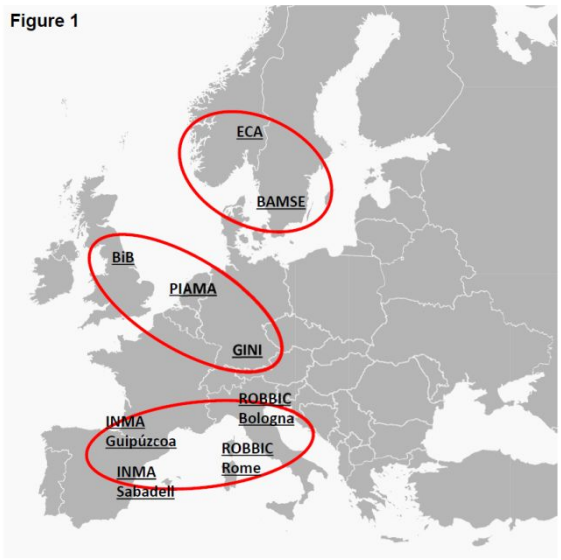


Figure 1. Regions covered by the analyzed MeDALL-birth cohorts. Names of the respective cohorts and red circles indicate the regions that were covered by the study populations.

Table I

| Cohorts | | Numbers of sera by age | | | | | | | Chip-versions used |
|---|---|---|---|---|---|---|---|---|---|
| Name | Country/Region | 1 y. | 4 yrs. | 8 yrs. (BAMSE) 7-9 yrs. (ROBBIC) | 10 yrs. | 12 yrs. | 15 yrs. | 16 yrs. | |
| 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 |

Table I. MeDALL-cohorts and numbers of samples analyzed with the MeDALL-chip. Samples obtained at 7-12 years (purple boxes) or at 15-16 years (yellow boxes) were combined in age groups.

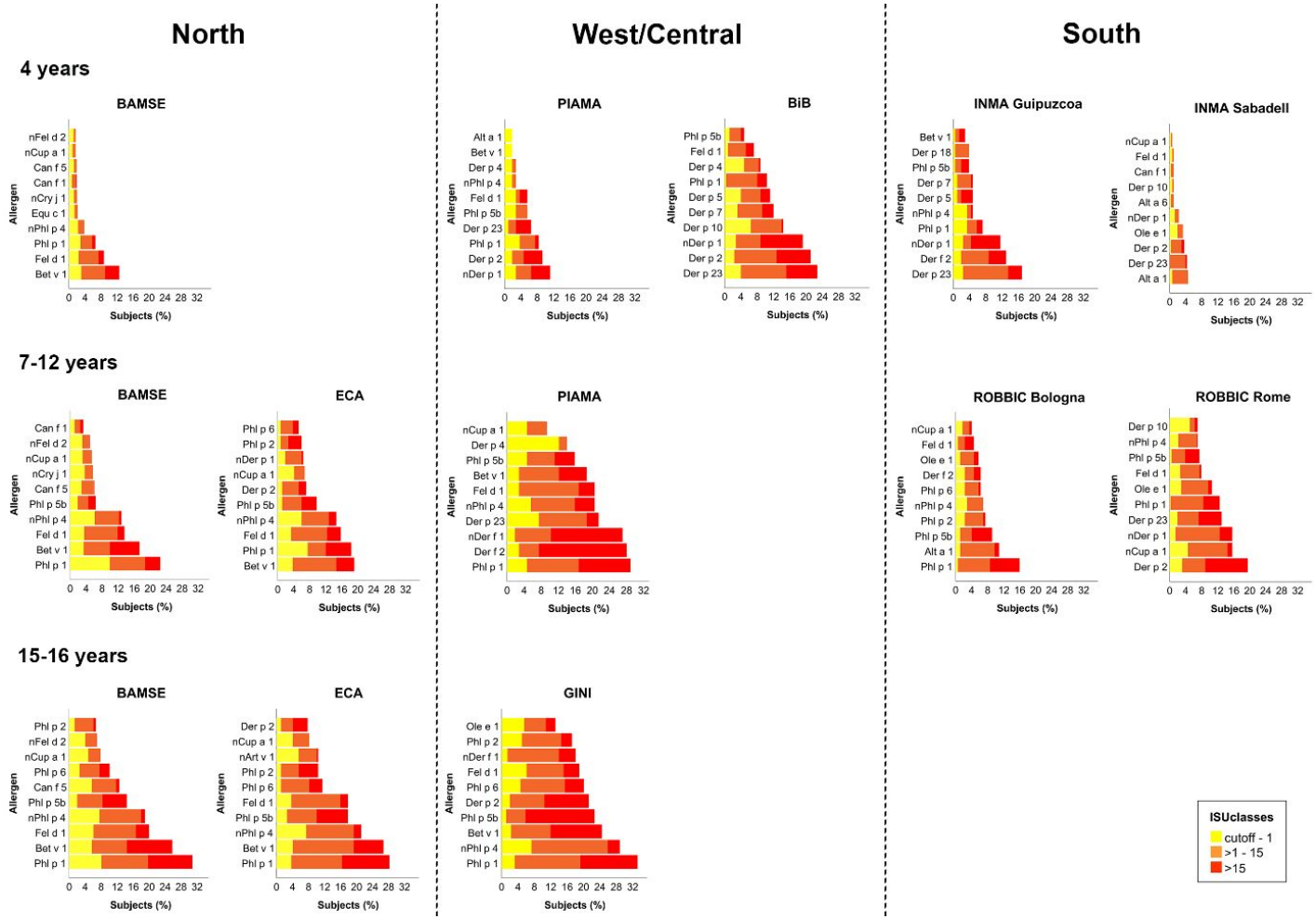


Figure 2. Overview of the 10 most frequently recognized primary respiratory allergens per cohort. The cohorts are organized based on age and region. For each cohort, allergens are ranked based on sensitization rate. Each bar shows the percentage of subjects with IgE levels within the different ISU classes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

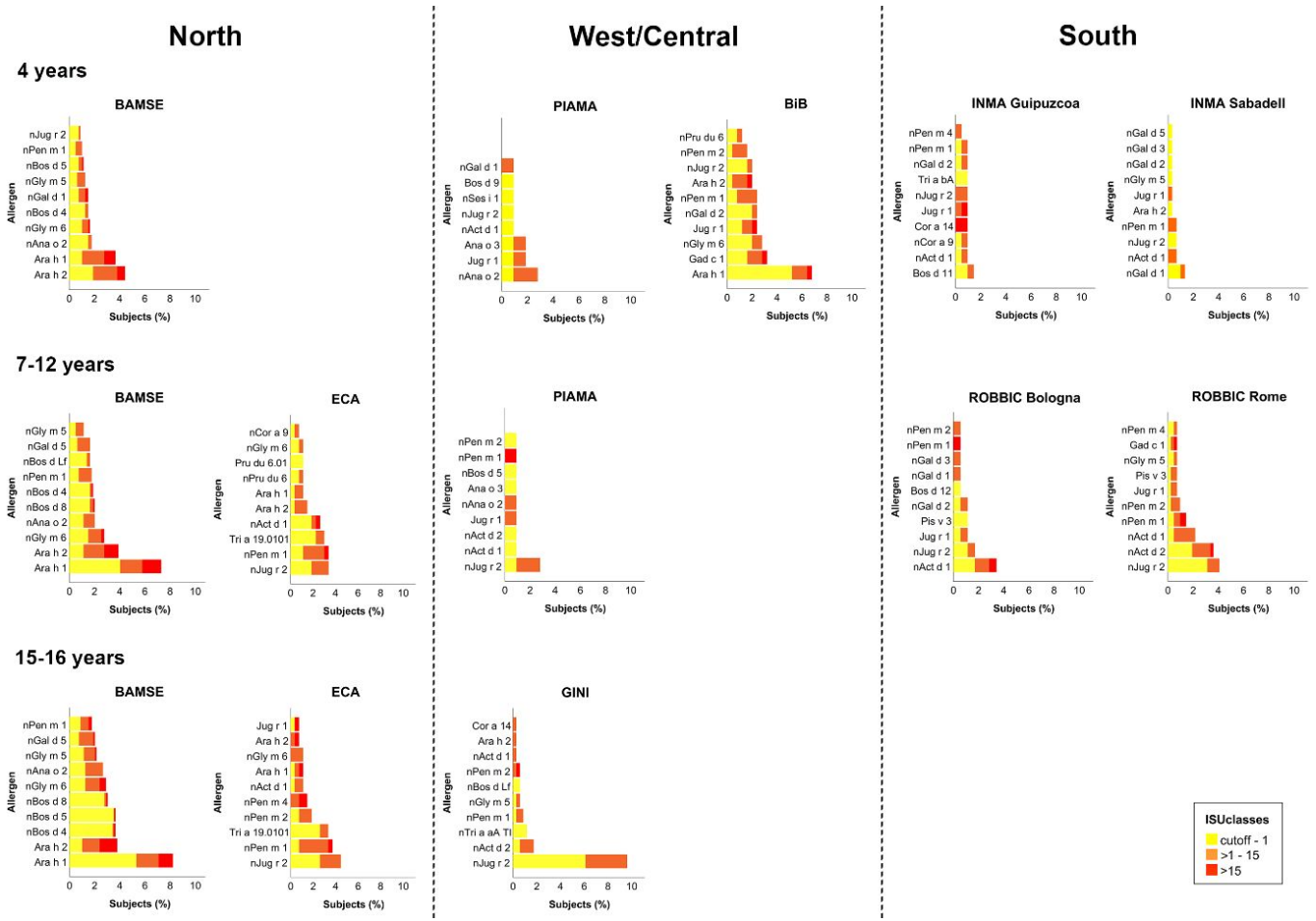


Figure 3. Overview of the 10 most frequently recognized type I food allergens per cohort. The cohorts are organized based on age and region. For each cohort, allergens are ranked based on sensitization rate. Each bar shows the percentage of subjects with IgE levels within the ISU classes (yellow = ≥0.3-1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

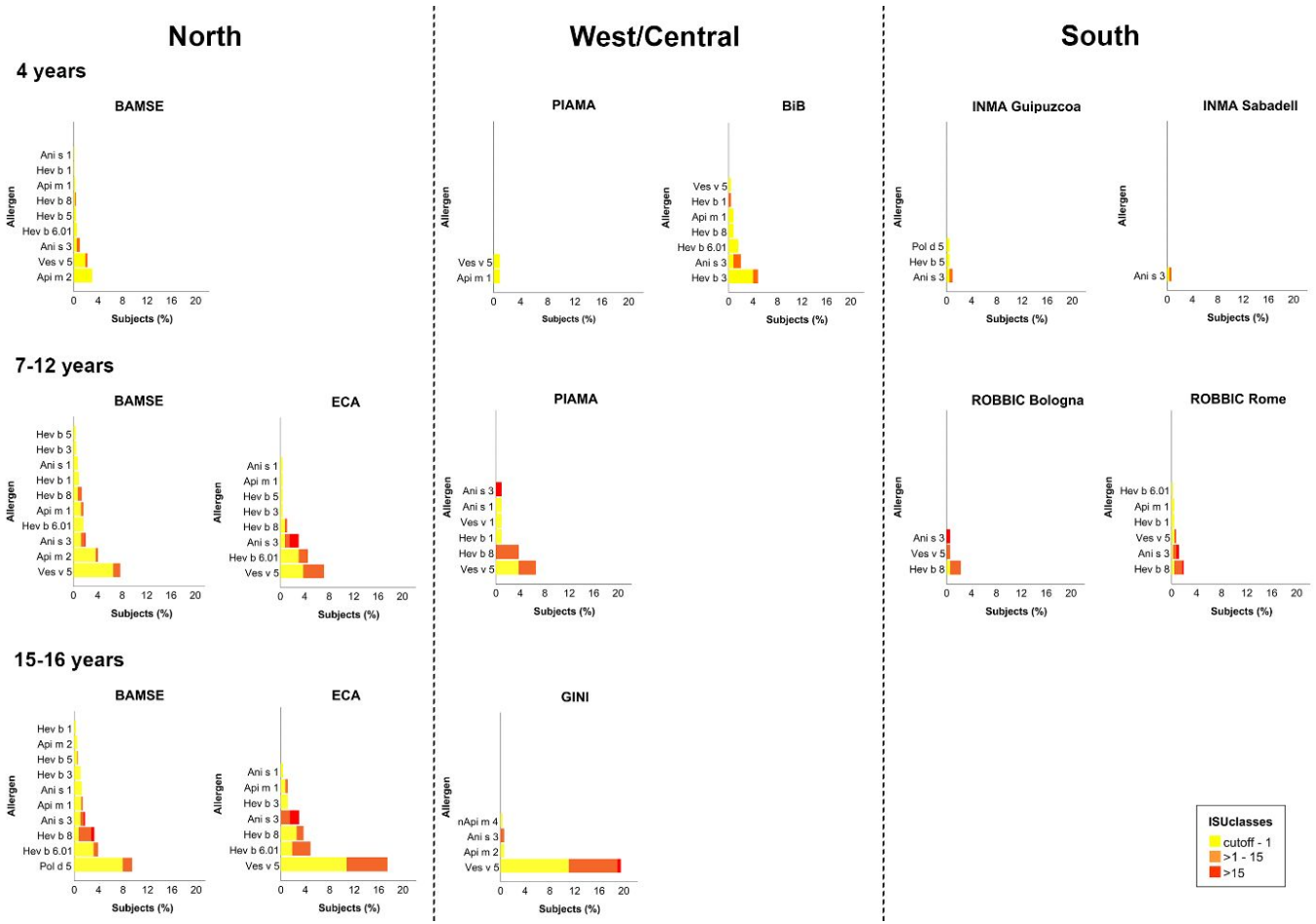


Figure 4. Overview of the 10 most frequently recognized primary other allergens per cohort. The cohorts are organized based on age and region. For each cohort, allergens are ranked based on sensitization rate. Each bar shows the percentage of subjects with IgE levels within the ISU classes (yellow =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

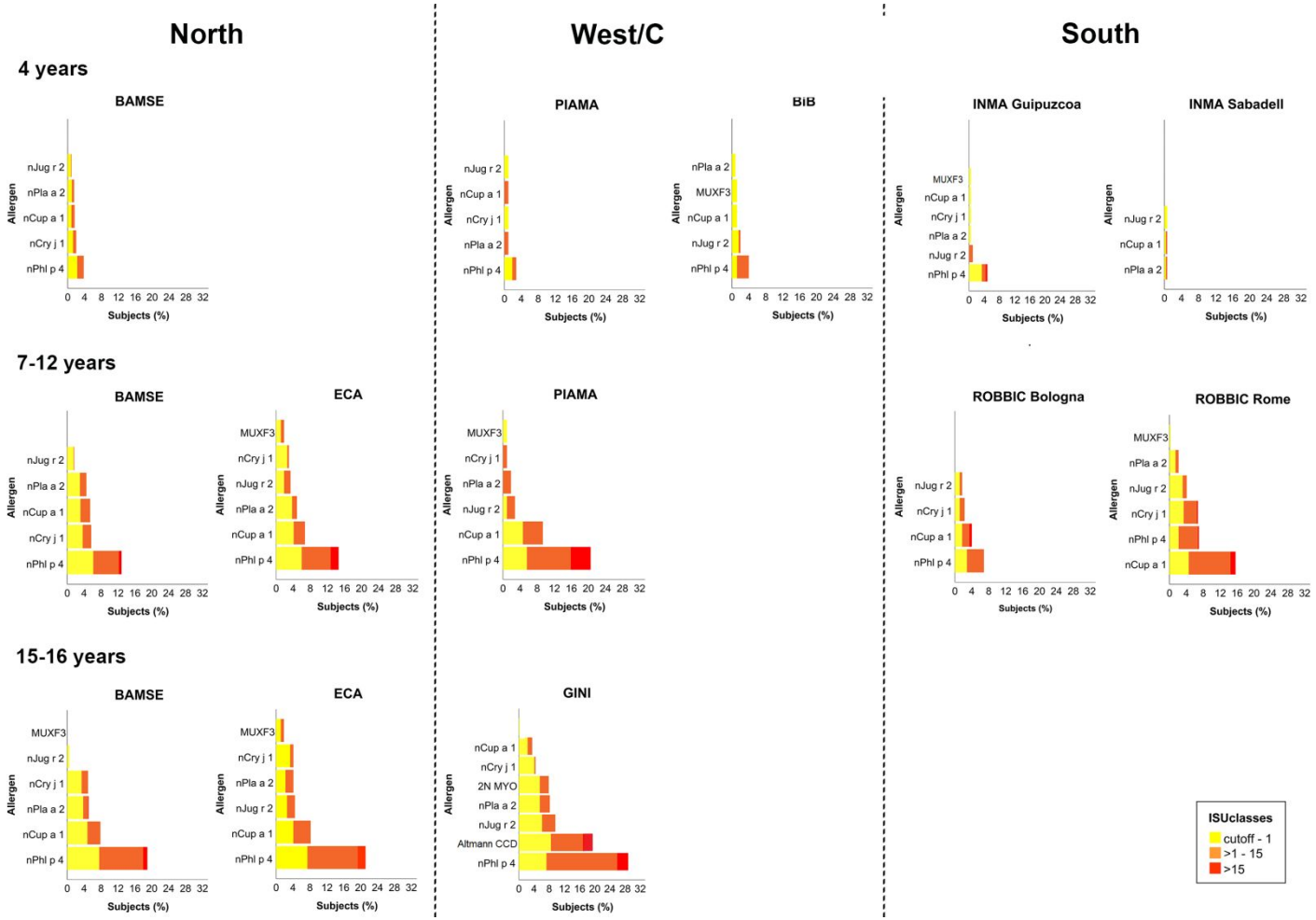


Figure 5. Overview of the most frequently recognized CCD-bearing allergens per cohort. The cohorts are organized based on age and region. For each cohort, allergens are ranked based on sensitization rate. Each bar shows the percentage of subjects with IgE levels within the ISU classes (yellow = ≥0.3-1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

# A molecular sensitization map of European children ~~followed from childhood to adolescence~~ reveals exposome- and climate-dependent sensitization profiles

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**Running title:** Molecular sensitization map of Europe

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**Abstract**

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

**Methods:** IgE sensitization profiles were determined in more than 2800 sera from children in 9 population-based cohorts in different geographical regions of Europe; north (BAMSE (Sweden), ECA (Norway)), west/central (PIAMA (the Netherlands), BiB (UK), 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 ~~in the same region~~ 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 ~~at all ages~~.

**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***,*** IgE-reactivity, Europe, exposome, MeDALL chip, sensitization profile

## 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](UK), 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 to 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 ~~followed from early childhood to adolescence~~.

## Materials and methods

### Cohorts and design of the study

IgE 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] (UK), 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 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[5-12]. The analysis of pseudonymised 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. ~~Randomly picked numbers of sera representative for each cohort were analyzed to avoid biases as much as possible.~~ Possible limitations of the study are mentioned in the discussion section. (https://www.strobe-statement.org/).

### 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 E1-E2).

## Data analysis

All analyses were performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA). 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 Determinants (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 designate protein allergens 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 E3-E6). 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).

## Results

### 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 four 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 years 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 ~~all regions. Serologically detectable IgE sensitization against at least one respiratory allergen increased from 3.7% at 1 year old (in PIAMA) to about 50% in the oldest age group (BAMSE, ECA and GINI) (Figure E1). Food allergens showed a less pronounced increase with age, with a prevalence of 9.3% at age 1 in the PIAMA cohort, which increased to about 15% at 15-16 years of age (BAMSE, ECA and GINI) (Figure E1) Sensitization via other routes (e.g., venom or latex allergy) occurred mostly at an older age. Around 15% of the 15-16 year olds (BAMSE, ECA and GINI) were sensitized to this "other" type of allergen (Figure E1). Despite this quantitative increase, n~~However, no major changes in the qualitative sensitization profiles (i.e., hierarchies of IgE sensitizations) were observed between different age groups (Figures 2-5).

### 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 p 1, 5b, 6, 2, 11 and 12 (Table E3). Phl p 1 was the ~~most~~ 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.

### 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 (Figure 2, Figure 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 E3, 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.

**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 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, Table E3). 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 E3). However, house dust mite allergens were only minor allergens in the northern cohort BAMSE at all ages. However, we also noted striking differences regarding sensitization to house dust mites in Southern Europe. In Sabadell which is close to Guipuzcoa ~~in the same region~~ 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).

**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 ~~primary~~ 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 E4).

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 (INMA Sabadell) at all ages, but mostly in less than 1% of the children (Table E4). Besides class food 1 allergens, cross-reacting PR-10 proteins like Cor a 10401, 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 E4).

**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 E5).

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.

**Sensitization to ~~carbohydrates is~~ CCD-bearing allergens is dominated by grass pollen nPhl p 4 in Northern, Central and Western Europe and by ~~cypress~~ 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 E6). Furthermore, the tree-derived CCD-bearing allergen Cup 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. ~~The only~~ A frequently recognized CCD-containing food allergen was the walnut allergen Jug r 2. It was ~~recognized~~ 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 E6).

## Discussion

This study provides the first comprehensive overview of ~~the onset and the development of~~ IgE-sensitization profiles at the molecular level in ~~a~~ 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.

~~Grass pollen allergen sensitization dominated in most of the European regions, while IgE reactivity to tree pollen was in a higher degree region-dependent. The major cat allergen, Fel d 1, was an important indoor allergen in almost all European regions in contrast to the house dust mite allergens Der p 1, 2 and 23 where the sensitization frequencies varied considerably between regions. Differences were also observed for class 1 food allergens. The major peanut allergen Ara h 1 was the most recognized primary allergen in the BiB cohort and second most recognized in the BAMSE cohort at 4 years, while in Southern and in Western/Central Europe, fruit allergens such as Pru p 3, the kiwi allergens Act d 1 and 2, but also egg allergens were among the most recognized allergen molecules. By contrast, sensitization to class 2 food allergens causing mainly oral allergy syndrome such as the Bet v 1-related PR10 allergens was tightly linked to primary sensitization to the corresponding respiratory allergens. Interestingly, 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.~~

~~While the timing of sensitization onset was very similar in the different European regions, many~~ Striking regional differences regarding molecular IgE sensitization profiles in the different cohorts were observed ~~could be observed in the recognition of allergens~~. There are only few previous studies which have analyzed molecular IgE sensitization profiles in population-based cohorts from individual countries (e.g., UK, Germany, Italy) which in fact confirm the molecular sensitization patterns which we observed for these countries[14, 15, 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 ~~have in an unambiguous manner shown that these differences can be explained by the~~ based on differences in the regional exposome. ~~E.g.~~ In fact, Siroux et al 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.[17] 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 ~~which are both located in the same region not far from each other in Spain~~[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 four 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 ~~most~~ 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~~, but~~ and hot ~~and Mediterranean~~ 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) ~~from~~ 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[26-30] 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[31], sensitization was observed in the INMA and ROBBIC cohorts. Cypress is another typical Mediterranean tree found above all in Italy[32] 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 HDM 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., HDM 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 ~~prominent~~ 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.[33] The data is in line with a report showing that a higher percentage of people in Norway, Sweden and the UK had a cat during childhood.[34] 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.[35] 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.[36, 37] This is in line with our finding that IgE to the house dust mite allergens were almost absent in BAMSE, ~~and~~ very low in Sabadell, present in ECA and PIAMA~~, but~~ and most prominent in the BiB cohort from the UK. 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 HDM allergens, like Der p 4, 5, 7 and 10.[38] Unlike house dust mites, the fungus *Alternaria alternata*, has shown to be an indoor allergen which grows better in a dry and warm climate.[22] 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[39]. 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.[40] 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.[41] 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.[42] Regarding peanut differences of allergen contact via the skin may also be considered to be responsible for different sensitization rates in the different populations ~~Sensitization against other food allergens depended mainly on~~ besides nutritional habits.[43] High sensitization rates to peanut allergens and to the major fish allergen Gad c 1 in the BiB cohort from UK 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.[44]

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.[45] 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.[46] 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 ~~difficult~~ 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 ~~from the same time points had not been collected in each of in the investigated cohorts~~ 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[47]. 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[48]. 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. ~~Nevertheless~~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 ~~and age-dependent~~ differences in IgE-reactivity profiles of the general populations, which depend largely on the local exposome and

climate. ~~Although there were different age groups no major changes in the sensitization profiles were noted.~~ 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.

**Authors contribution:**

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, K L-C, DP, DG, HAS, RB, UG, MS, JH, DW, JW, SV, SW, AB collected patients' material and/or prepared and characterized allergen molecules. CL, GK, MvH, RV contributed to data interpretation and wrote the first draft of the manuscript. All authors critically reviewed the manuscript and approved the submitted version.

**Conflicts of interest:** 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.

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## Figures and legends

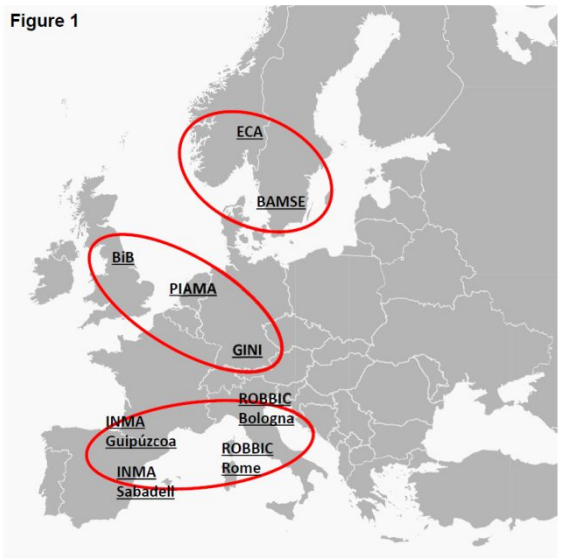


Figure 1. Regions covered by the analyzed MeDALL-birth cohorts. Names of the respective cohorts and red circles indicate the regions that were covered by the study populations.

Table I

| Cohorts | | Numbers of sera by age | | | | | | | Chip-versions used |
|---|---|---|---|---|---|---|---|---|---|
| Name | Country/Region | 1 y. | 4 yrs. | 8 yrs. (BAMSE) 7-9 yrs. (ROBBIC) | 10 yrs. | 12 yrs. | 15 yrs. | 16 yrs. | |
| 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 |

Table I. MeDALL-cohorts and numbers of samples analyzed with the MeDALL-chip. Samples obtained at 7-12 years (purple boxes) or at 15-16 years (yellow boxes) were combined in age groups.

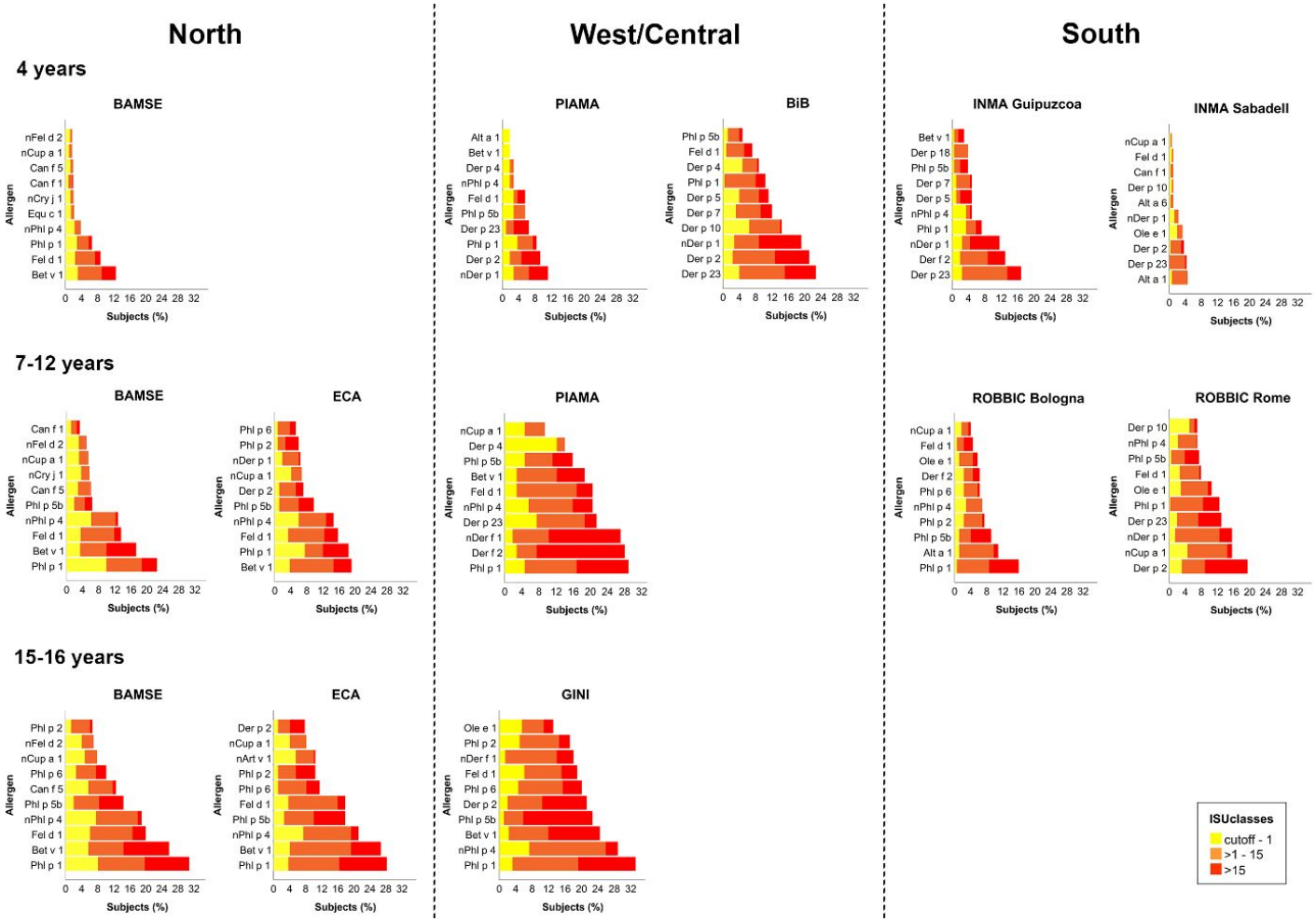


Figure 2. Overview of the 10 most frequently recognized primary respiratory allergens per cohort. The cohorts are organized based on age and region. For each cohort, allergens are ranked based on sensitization rate. Each bar shows the percentage of subjects with IgE levels within the different ISU classes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

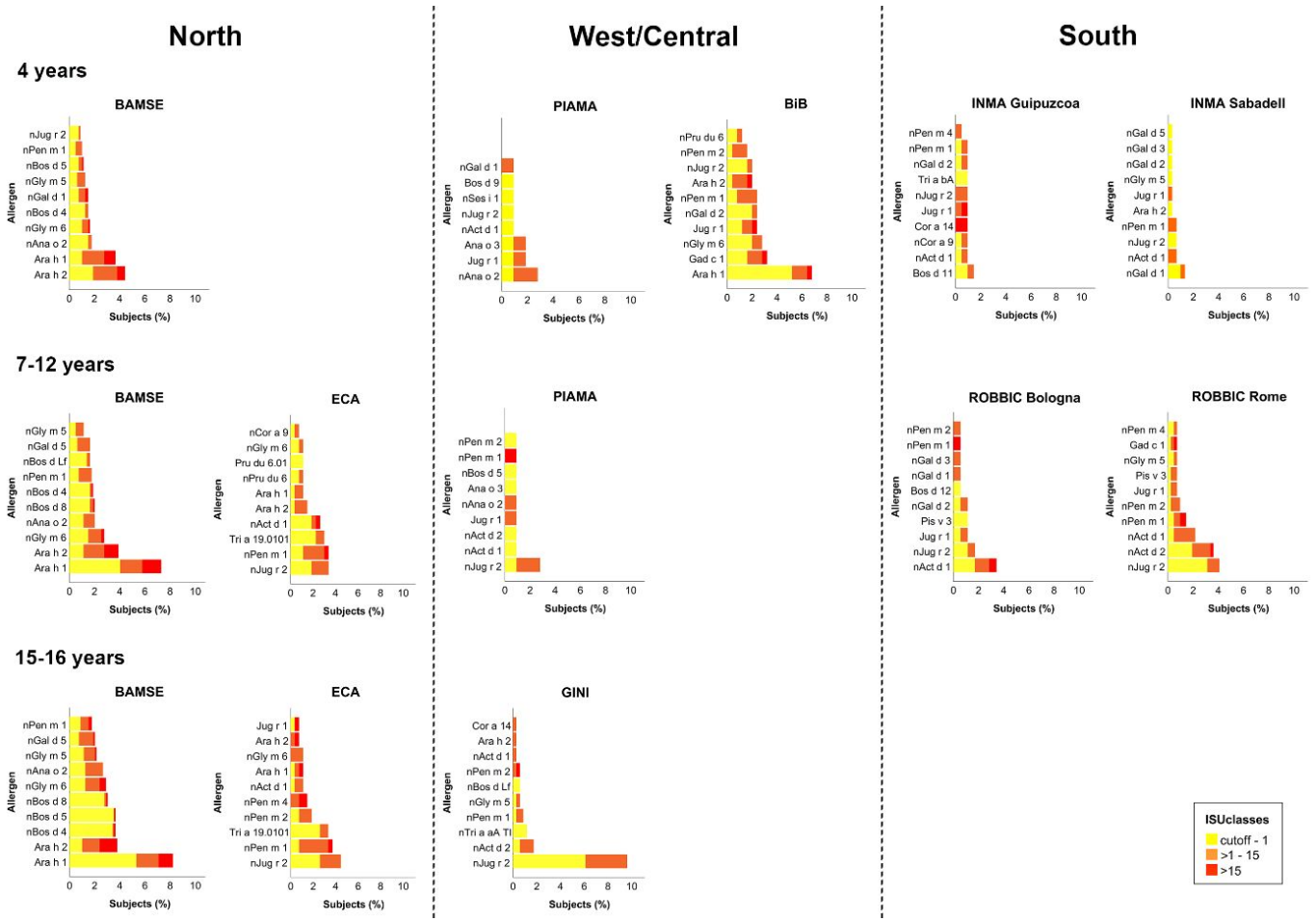


Figure 3. Overview of the 10 most frequently recognized type I food allergens per cohort. The cohorts are organized based on age and region. For each cohort, allergens are ranked based on sensitization rate. Each bar shows the percentage of subjects with IgE levels within the ISU classes (yellow = ≥0.3-1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

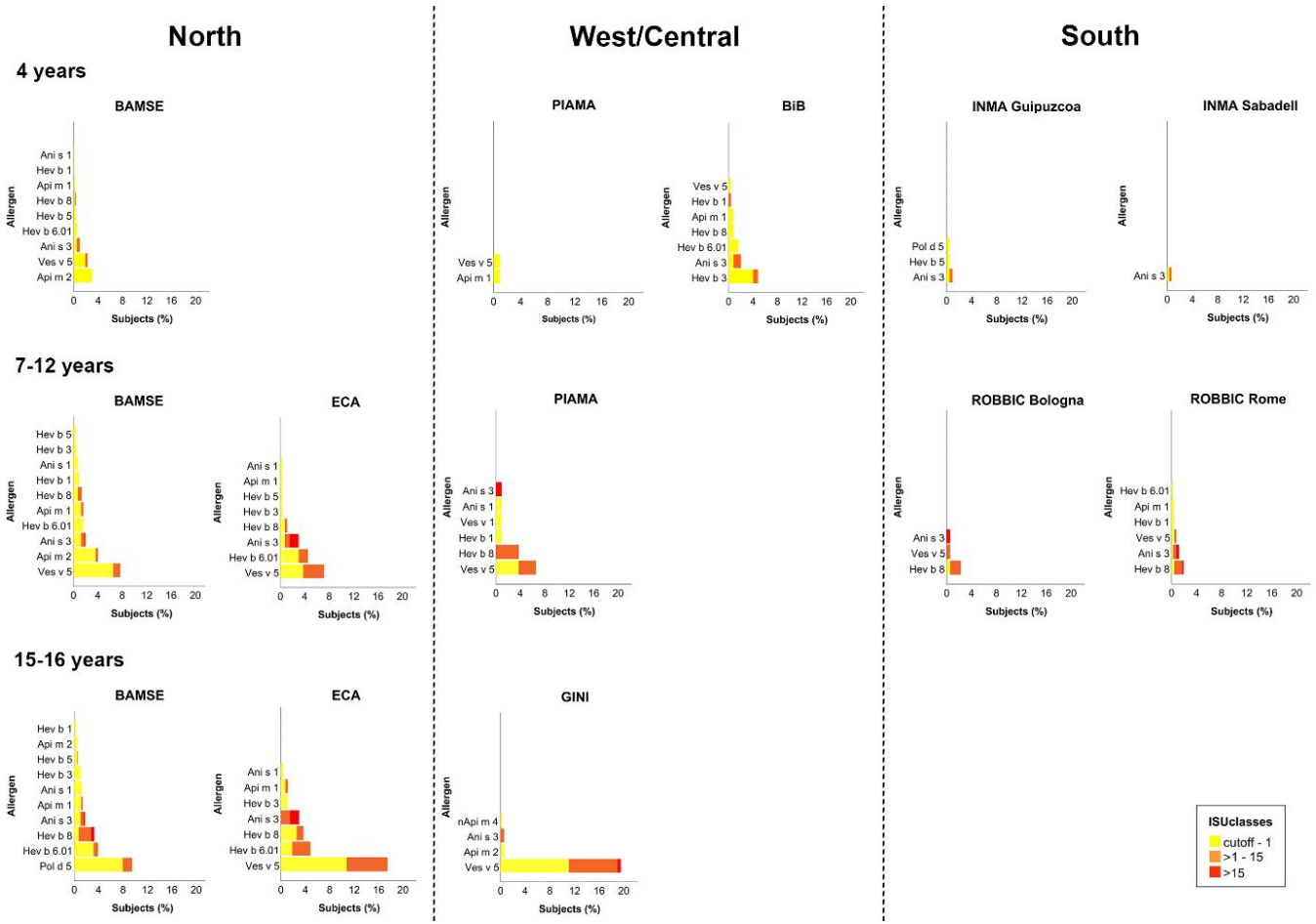


Figure 4. Overview of the 10 most frequently recognized primary other allergens per cohort. The cohorts are organized based on age and region. For each cohort, allergens are ranked based on sensitization rate. Each bar shows the percentage of subjects with IgE levels within the ISU classes (yellow =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

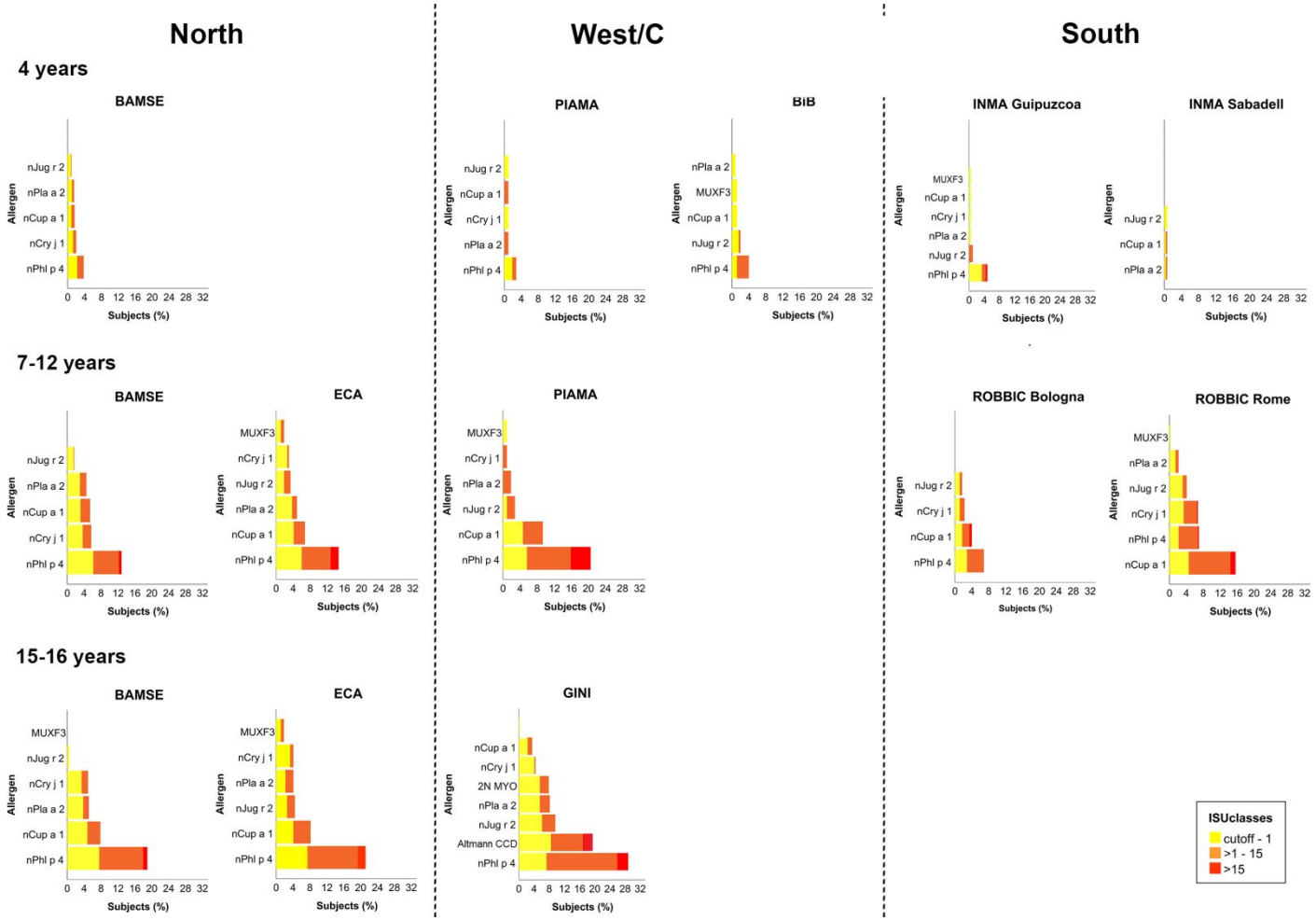


Figure 5. Overview of the most frequently recognized CCD-bearing allergens per cohort. The cohorts are organized based on age and region. For each cohort, allergens are ranked based on sensitization rate. Each bar shows the percentage of subjects with IgE levels within the ISU classes (yellow = ≥0.3-1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

**Supplementary file 1**

# A molecular sensitization map of European children followed from childhood to adolescence reveals exposome- and climate-dependent sensitization profiles

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## Methods

### Production of MeDALL-chips

Compared to the commercial version of the allergen-chip, i.e., ImmunoCAP ISAC, more than 60 additional allergen molecules were added, and a different coupling chemistry was applied, increasing the sensitivity of the MeDALL-chip.[1] In the course of the MeDALL-project, from December 2010 until May 2015, the array layout was subjected to some minor modifications (Tables E1 and E2).

### Quality assessment

To assess background signals, median signal intensity of the negative control was calculated for each allergen. In addition, individual signals of the buffer control that were higher than the cut-off, i.e., ≥ 0.3 ISU-E, were identified and re-evaluated on the original scans of the respective microarrays in order to rule out artifacts. If the negative control showed a median background activity ≥ 0.3 ISU-E, the cut-off level was increased by the factor of three for the respective allergen or, if background levels exceeded 0.9 ISU-E, this allergen was excluded from analysis for the respective cohort (Table E2).

To identify potential background activity of serum samples with individual allergen molecules, allergens with a median signal intensity > 0 ISU were re-assessed regarding (1.) co-occurrence with positivity to other, non-cross-reactive allergens on the array which would have been indicative of carry-over during microarray-spotting, (2.) differences in rates of positivity between individual test runs of the same cohort and, (3.) for cohorts with samples collected at several ages of the children, if in the same subjects an increase of signal intensity over time was detected. If, according to those criteria, particular allergens showed background signals potentially leading to false positive results, the respective samples were either re-tested or, if this was not possible due to lack of sample volume, the cut-off was increased by the factor of three. If false positive results still could not be eliminated by these approaches, the respective allergens were not considered for analysis for the respective cohort.

The calibrator serum showed IgE-reactivity with approximately 2/3 and IgG-reactivity with almost all spotted allergens on the MeDALL-chip. Therefore, in addition to the quality control performed by the producer of the microarrays (Thermo Fisher/Phadia AB), antibody reactivity of spotted allergens was confirmed by using the calibrator serum. Allergens that showed lack of reactivity were excluded from analysis for the respective cohort. Likewise, eventual batch-to-batch differences in signal intensities were compensated using results obtained with the control serum.

**Detection of allergen-specific IgE using the MeDALL-microarray**

Serum aliquots (mostly 50-100 µl per sample) were sent on dry ice to the Department of Pathophysiology and Allergy Research (Vienna, Austria) for establishing IgE-reactivity profiles by microarray. Samples were stored at -20°C and thawed immediately prior to analysis. Sera were tested for IgE against over 170 proteins on the customized allergen microarray as described previously.[1] In brief, the slides were washed in wash buffer (Thermo Fisher/Phadia AB, Uppsala, Sweden) using a glass staining jar and a magnet stirrer for 5 minutes. Then, after drying by centrifugation (1000 xg, 1 minute, room temperature), 35 µl of undiluted serum were applied and incubated in a humid chamber at room temperature with gentle rocking for 2 hours. Slides were quickly rinsed using a spray bottle and immediately immersed in wash buffer, followed by a second wash step as above. For detection, 35 µl of a fluorochrome labelled anti-IgE detection conjugate (Thermo Fisher/Phadia AB) was added and incubated for 30 minutes as described before. After a final wash step, the microarrays were scanned on a LuxScan-10 K microarray scanner (Capital-Bio, Beijing, People's Republic of China). Scans were evaluated by Microarray Image Analyzer v3.1.2 software (Thermo Fisher/Phadia AB). IgE-levels ≥ 0.3 ISU-E were defined as positive, in accordance with the cut-off level of ImmunoCAP ISAC (Thermo Fisher/Phadia AB). For calibration and compensation of possible batch-to-batch differences, an aliquot of the same calibrator serum, i.e. a serum pool reactive with most allergen molecules on the microarray, was included in each run during the complete MeDALL project. In addition, a buffer control (Sample Diluent for ImmunoCAP IgG/IgA, Thermo Fisher/Phadia AB) was used to check for non-specific binding of the detection antibody to the chip-surface or to particular allergen molecules (see below).

**Sample collection and ethics**

From all cohorts, sera were randomly picked, therefore representing the general population. For each cohort, approval by the respective institutional review board was given for performing the analyses described within the scope of the MeDALL-project. Pseudonymized sera were analyzed at the Medical University of Vienna with permission of the Ethics committee of the Medical University of Vienna, EK1640/2004.

**Figures and tables**

**Figure E1.** Overall sensitization rates per age group for each sensitization route.

**Table E1.** Allergens on the different versions of the MeDALL-microarray. Marker allergens are highlighted by green boxes (column "Allergen"), natural allergens by gray boxes (column "Rec. of natural"), CCD-bearing glycoproteins by yellow boxes (column "CCD"). Column "Chip-version" shows which allergens were represented on the different versions of the MeDALL-chip (marked by an "x").

**Table E2.** Adjustment of IgE-values, cut-off level and exclusion of allergens from analysis for technical reasons.

**Table E3.** Numbers, percentages and IgE levels of sera positive to respiratory allergens on the MeDALL-chip, shown for each age group for the different cohorts.

**Table E4** Numbers, percentages and IgE levels of sera positive to food allergens on the MeDALL-chip, shown for each age group for the different cohorts.

**Table E5.** Numbers, percentages and IgE-levels of sera positive to other allergen molecules on the MeDALL-chip.

**Table E6.** Numbers, percentages and IgE-levels of sera positive to CCD-markers and CCD-bearing natural allergen molecules on the MeDALL-chip.



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