

**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, personalized 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.

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 added 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)

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3 The manuscript describes a collaborative analysis of sera from seven
4 allergy birth cohorts in Europe, a remarkable international effort. The
5 questions asked are important and have not been described before. They
6 provide evidence that IgE mediated sensitivity to a variety of allergens
7 differs in populations living in different parts of Europe, some in
8 predictable ways, others that are surprising. The authors have been drawn
9 careful conclusions well within their data. I only have minor suggestions
10 that might clarify the presentation.
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21 **Reply:** We thank the reviewer for the excellent summary of our study and the kind comments.
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25 Abstract keywords – spelling of “personalized” should be corrected
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27 **Reply:** Corrected to “personalised”. **Line 77** of revised manuscript.
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31 Ln 143- The fact that sera were analyzed in a single central laboratory is
32 an important point to my mind, greatly strengthening the quality of the
33 data. In addition to the detailed methods described in the online
34 repository, I would suggest a sentence in the methods, such as: “Serum
35 aliquots were sent to the Department of Pathophysiology and Allergy
36 Research (Vienna, Austria) for establishing IgE-reactivity profiles by
37 microarray.”
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45 **Reply:** We thank the reviewer for pointing this strength of our study out and gladly followed
46 the suggestion. **Lines 139-148** of the revised manuscript.
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50 Ln 297 – Here and in later parts of the manuscript, the source
51 description should be moved to the reference section and given an
52 appropriate reference number in the text.
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3 **Reply:** We thank the reviewer for this suggestion and implemented it. The source descriptions
4 have obtained reference numbers and were listed in the references. See [lines 326-327](#) of
5 revised manuscript.
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10 Ln 313 “most dominant” should be simply “dominant”

11 **Reply:** Corrected as suggested, see [line 333](#) of revised manuscript.
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16 Ln330 – how is “most prominent “ related to “most dominant”

17 This should reconcile for clarity

18 **Reply:** We agree and corrected to “most frequently recognized”. See [line 365](#) of revised
19 manuscript.
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25 Ln335- indoor cat allergen ref 25 check

26 **Reply:** We thank the reviewer for the insightful comment and checked the reference. In fact,
27 reference 25 says that cat allergen levels are lower in Spain than in middle Europe and thus
28 seems suitable. The paper also says “that not having a cat in the home is associated with
29 substantially lower Fel d 1 concentration, but does not protect against high Fel d 1 exposure in
30 communities where cat ownership is common.” However, the latter statement does not seem
31 to contradict our statement that cat allergen levels are lower in southern countries.
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36 Ln341 – since the manuscripts data described presence of IgE antibody in
37 sera, not home allergen levels, this sentence should read: “This is in
38 line with our finding that IgE to house dust mite allergens was almost
39 absent in sera from the BAMSE cohort, very low in sera from the Sadabel
40 cohort”
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45 **Reply:** We thank the reviewer and corrected the sentence, see [line 376-377](#) of the revised
46 manuscript.
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49 Ln387 -I do not understand the caveat that sera were not collected at the
50 same time. According to Table 1, and subsequent figures, adequate samples
51 were available to support the conclusion that there were regional
52 differences. Perhaps they mean that the start dates for the various
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3 cohorts were spread over time, so that they could not compare seasonal
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5 differences that might relate to “wet or dry” years or temporal
6
7 changes that might relate to climate change. The authors should be
8
9 specific.

10
11 **Reply:** We thank the reviewer. In fact we meant that children from the different cohorts did not
12 have exactly the same age (see Table 1, BAMSE: 8 years; ROBBIC: 7-9 years; ECA: 10 years;
13 PIAMA: 12 years). We have corrected our statement to make this more clear, see [lines 433-434](#)
14 of revised manuscript.
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17 **Reviewer: 2**

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19 COMMENTS FOR THE AUTHOR(S)

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21 From multiple birth cohorts across Europe the dominant sensitizations at
22
23 each age have been defined. These sensitizations are discussed in the
24
25 context of exposures due to climate and other factors.
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27 **Reply:** We thank the reviewer for the concise summary.
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30
31 A few minor comments for consideration

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33 1) The reader may expect and desire a map of sensitization. Perhaps it may
34
35 be possible to have a graphic (similar to the Fig 1 map showing the
36
37 cohorts) but with the results. I appreciate there are many results and
38
39 they differ by age. Perhaps the sensitizations at each age group (Fig 5)
40
41 could be overlaid onto fig 1 or the areas exploded to make room.
42

43 **Reply:** We thank the reviewer for this excellent comment. In fact we would have wished to
44 draw a relatively simple overview of the molecular IgE sensitization profiles according to
45 regions as suggested by the reviewer. However, it turned out that we found considerable
46 differences in molecular IgE sensitization profiles in two regions of Spain and Italy which are
47 relatively close to each other (i.e., Rome and Bologna, Italy; Sabadell and Gipuzkoa, Spain). It
48 thus turns out that differences in molecular IgE reactivity profiles can vary strongly even in the
49 same country. We have revised our manuscript to make this point more clear and hope for the
50 understanding of the reviewer that we could not find a way to present the complex data in the
51 simplified way as requested by the reviewer.
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55 2) the citation of the web link say be more readable as a citation with the
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3 Internet address in the reference list at the end
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5 **Reply:** We completely agree with the reviewer and provided reference numbers for the
6 internet links. See [lines 323-325](#) of the revised manuscript.
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10 **Reviewer: 3**

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12 **COMMENTS FOR THE AUTHOR(S)**

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14 The purpose of characterizing patients based on molecular investigations
15 is fundamental and indispensable for a correct diagnosis. However, some
16 limitations appear in this study. It is not possible to compare the
17 increase with age of the allergic sensitization in different population if
18 the patients were not examined at the same time. This manuscript contains
19 important information, I suggest to rewrite it.
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26 **Reply:** We thank the reviewer for the comment and must agree. It is indeed not possible to
27 compare the increase of IgE sensitizations between the different cohorts because sera were
28 obtained at different time points and for certain cohorts at only one time point. Only for certain
29 cohorts we could make statements about the age-dependent evolution of IgE sensitizations.
30 We have revised the manuscript accordingly. See [lines 1-3, 119, 125, 187-194, 212, 346-351,](#)
31 [459-460](#) of the revised manuscript.
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37 Table 1 and figure 2. Not all children have been followed from childhood
38 to adolescence. Therefore, the comparison between the various populations
39 is uncertain. Considering this, the title of the manuscript should be
40 modified.
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44 **Reply:** Again we must agree with the reviewer and rewrote the title “A molecular sensitization
45 map of European children reveals exposome- and climate-dependent sensitization profiles”.
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50 Authors should explain how the sera were randomly picked. The sera from 9
51 larger population-based cohorts, located in different geographical regions
52 of Europe, were previously studied for atopic disease. More information
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3 should be provided. Authorizations to employ the sera should be provided
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5 by local ethics committees and caregivers.
6

7 **Reply:** We thank the reviewer for the valuable comments. Sera were randomly picked within
8 each cohort taking into consideration that only sera from children who were born in the region
9 and spent at least the first year of life there were analysed. Furthermore, we aimed at a gender
10 balance regarding the samples. In those cohorts where different time points were analysed sera
11 were taken from children for whom samples were available at each of the time points studies.
12 For each of the cohorts ethics approval and written informed consent from the parents or legal
13 guardians of the children was available for the analysis of allergen-specific IgE (see references
14 5-12). The analysis of pseudonymised serum samples was performed at the Department of
15 Pathophysiology and Allergy Research, Medical University of Vienna, Austria in a centralized
16 manner with permission of the Ethics committee of the Medical University of Vienna,
17 EK1641/2014. This information was included in the main revised manuscript, [lines 139-148](#).
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24 Page 12 line 172- 175. It is not possible to compare the increase with age
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26 of the allergic sensitization in different population if they were not
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28 examined at the same time, e.g., PIAMA population vs BAMSE, ECA and GINI
29
30 population were not examined when they were one year old.
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34 **Reply:** We agree with the reviewer and limited our statement to those cohorts where follow up
35 samples were available. See [lines 1-3, 119, 125, 187-194, 212, 346-351, 459-460](#) of the revised
36 manuscript.
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41 Page 10. Authors should explain why sensitization to Cup a 1 in southern
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43 population should be due to CCD and not to the Cup a 1 pectate lyase.
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45 According to literature data, Cup a 1 is one of the main allergens in Rome
46
47 (Italy) Pag 18. The sentence “other cohorts reactivity to nJug r 2 was
48
49 paralleled by an increase of IgE to Jug r 1, indicative of genuine
50
51 IgE-sensitization to walnut” should be better explained. The presence of
52
53 the cross-reactive carbohydrate determinants (CCD) makes it difficult to
54
55 draw firm conclusions on any association. Walnut contains more than one
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3 allergen and Jug r 2 and Jug r 1 are different seed storage proteins. One
4 patient might be sensitized to Jug r 1 and to Jug r 2 CCD but he could be
5 also sensitized to the 7s vicilin Jug r 2. Rather than looking for an
6 association with different molecules coming from walnut, it would be
7 better to look on the chip for the sensitization to other 7s vicilin
8 coming from different sources.
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17 **Reply:** The reviewer makes again a very good comment. It is indeed not possible to use natural
18 CCD-bearing allergens as CCD markers because IgE antibodies may be directed to their peptide
19 epitopes and/or CCDs to a varying degree. We therefore focused on IgE positivity to the CCD
20 marker MUXF3 and clarified in the manuscript that the terms MUXF3 and CCD marker are
21 identical because only MUXF3 (Ana c 2.0101) was present on all chip versions (see Table E1). If
22 one defines CCD-positivity according to MUXF3 sensitization the percentage of CCD-positive
23 subjects is quite comparable among the cohorts. The frequency of IgE-positivity to CCD-bearing
24 allergens may only serve as an indicator what CCD-bearing allergen may have been responsible
25 for inducing CCD-specific IgE in addition to the peptide-specific IgE. We have carefully revised
26 the manuscript with the goal to avoid the misunderstanding that the term “CCD-bearing
27 allergen” may be confused with the term CCD marker (i.e., MUXF3). See [lines 165-168](#) of
28 revised manuscript. Following the reviewers suggestion to look for associations of IgE
29 reactivities to 7s vicilins we were not successful to find such associations regarding the 7s
30 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
31 may be or is probably due to the fact that the sequence homologies among these allergens are
32 not very high.
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41 The nomenclature of the table E6 should be made uniform with the name of
42 the exact CCD marker present on the chip which instead is sometimes named
43 “marker” and other times as MUXF3, other times it is missing (BAMSE,
44 PIAMA, INMA, ROBBIC). Additionally, Authors should discuss the low
45 percentage of sensitization towards MUXF3 compared to other CCD bearing
46 proteins since this protein is considered one of the main markers of CCDs.
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3 **Reply:** Following the reviewers suggestion we corrected Table E6 to make clear that MUXF3
4 was meant (see revised Table E6). As already indicated above we have revised the manuscript
5 to address the discrepancies of IgE recognition frequencies to CCD-bearing allergens and the
6 CCD marker MUXF3 see: “It is indeed not possible to use natural CCD-bearing allergens as CCD
7 markers because IgE antibodies may be directed to their peptide epitopes and/or CCDs to a
8 varying degree. We therefore focused on IgE positivity to the CCD marker MUXF3 and clarified
9 in the manuscript that the terms MUXF3 and CCD marker are identical because only MUXF3
10 (Ana c 2.0101) was present on all chip versions (see Table E1). We have tested other CCD
11 markers (i.e., 2N MYO, Altmann’s CCD blocker as indicated in Table E1) only in the GINI cohort.
12 If one defines CCD-positivity according to MUXF3 sensitization the percentage of CCD-positive
13 subjects is quite comparable among the cohorts. The frequency of IgE-positivity to CCD-bearing
14 allergens may only serve as an indicator what CCD-bearing allergen may have been responsible
15 for inducing CCD-specific IgE in addition to the peptide-specific IgE. We have carefully revised
16 the manuscript with the goal to avoid the misunderstanding that the term “CCD-bearing
17 allergen” may be confused with the term CCD marker (i.e., MUXF3).” **Lines 422-432.**
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26 Figure E1. This figure might be misleading. It does not seem correct to
27 state that this figure reports the sensitization rate per age group for
28 each sensitization route. Not all children of the examined population were
29 followed from childhood to adolescence.
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33 **Reply:** We agree with the reviewer and deleted Figure E1 and the corresponding text as it is
34 indeed misleading and built on incomplete data from different cohorts.
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39 **Reviewer: 4**

40 **COMMENTS FOR THE AUTHOR(S)**

41 The current manuscript provides IgE sensitization data obtained from 2800
42 sera. The chip based study was performed within MeDALL. The serum samples
43 were selected from different birth cohort studies (BAMSE, ECA, PIAMA, BiB,
44 GINIplus, INMA and PIAMA and ROBBIC). This approach enables access to a
45 large number of serum samples and allows comparing different exposure
46 scenarios. The authors state: “These sera have been randomly picked,
47 therefore representing the general population.” This needs a more
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3 detailed description since the different prospective cohort studies had
4 applied different inclusion criteria and different primary goals ranging
5 from identifying risk factors for allergic diseases, assessing onset of
6 allergic manifestations, genetic predisposition up to assessing different
7 nutritional habits, ethnicities, and environmental pollution. Therefore
8 the samples might be heterogeneous and may affect the conclusions drawn
9 from these findings.
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17 **Reply:** We thank the reviewer for the valuable comments. Sera were randomly picked taking
18 into consideration only that children were born in the region and spent at least the first year of
19 life there. Furthermore, we aimed at a gender balance regarding the analysed sera. In the
20 cohorts where different time points were analysed sera were taken from children for whom
21 samples were available at all time points analysed. Our approach is thus indeed a quite random
22 approach because it only takes gender balance and allergen exposure in the given area into
23 account. However, we have discussed that factors such as atopic background of the parents
24 could be a limitation of our study. Following the reviewers recommendation we included the
25 fact that we did not make a selection according to genetic background of children, nutritional
26 habits, ethnicities and environmental pollution, which, as we have shown earlier may have a
27 modest, if any, effect (Air pollution and IgE sensitization in 4 European birth cohorts-the
28 MeDALL project. Melén E, Standl M, Gehring U, Altug H, Antó JM, Berdel D, Bergström A,
29 Bousquet J, Heinrich J, Koppelman GH, Kull I, Lupinek C, Markevych I, Schikowski T, Thiering E,
30 Valenta R, van Hage M, von Berg A, Vonk JM, Wickman M, Wijga A, Gruzieva O. J Allergy Clin
31 Immunol. 2021 Feb;147(2):713-722. doi: 10.1016/j.jaci.2020.08.030. Epub 2020 Sep 11) as
32 additional limitations of our study (see [lines 445-455](#) of the revised manuscript). On the other
33 hand we think that the approach of analysing randomly picked samples has also strengths as it
34 provides a real-life snapshot of the molecular IgE sensitization profiles in different regions and
35 the samples sizes were not too small and surely provided reliable results regarding the
36 molecular IgE sensitization profiles.
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46 The chip based approach is sound and well done and
47 supported by an already commercially available product. Another limitation
48 of the current manuscript is the lack of discussion comparing the obtained
49 data with data from other studies such as e.g. EUROPREVALL and others.
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3 **Reply:** We thank the reviewer for pointing out the technical strength of our allergen
4 microarray. We agree that it is appropriate to make comments regarding other studies. In fact
5 the EuroPrevall study has first focused on food allergic subjects and not investigated random
6 population samples especially not of children with the same age from a particular region.
7 EuroPrevall has also not performed a comprehensive analysis of molecular IgE sensitizations
8 against large panels of respiratory allergens, food allergens, other allergens such as venoms and
9 carbohydrates at the same time (The EuroPrevall surveys on the prevalence of food allergies in
10 children and adults: background and study methodology. Kummeling I, Mills ENC, Clausen M,
11 Dubakiene R, Pérez CF, Fernández-Rivas M, Knulst AC, Kowalski ML, Lidholm J, Le TM, Metzler
12 C, Mustakov T, Popov T, Potts J, Van Ree R, Sakellariou A, Töndury B, Tzannis K, Burney P.
13 Allergy. 2009 Oct;64(10):1493-1497. doi: 10.1111/j.1398-9995.2009.02046.x. Epub 2009 Apr 6).
14 We have mentioned this in our revised discussion (see [lines 383-386](#)). There are only a few
15 other studies performed with smaller panels of allergen molecules which are limited to certain
16 areas in Europe (e.g, UK: Evolution pathways of IgE responses to grass and mite allergens
17 throughout childhood. Custovic A, Sonntag HJ, Buchan IE, Belgrave D, Simpson A, Prosperi MCF.
18 J Allergy Clin Immunol. 2015 Dec;136(6):1645-1652.e8. doi: 10.1016/j.jaci.2015.03.041. Epub
19 2015 May 8; Germany: Evolution of the IgE and IgG repertoire to a comprehensive array of
20 allergen molecules in the first decade of life. Huang X, Tsilochristou O, Perna S, Hofmaier S,
21 Cappella A, Bauer CP, Hoffman U, Forster J, Zepp F, Schuster A, D'Amelio R, Wahn U, Keil T, Lau
22 S, Matricardi PM. Allergy. 2018 Feb;73(2):421-430. doi: 10.1111/all.13269. Epub 2017 Oct 9 and
23 Italy: Cross-sectional survey on immunoglobulin E reactivity in 23,077 subjects using an
24 allergenic molecule-based microarray detection system. Scala E, Alessandri C, Bernardi ML,
25 Ferrara R, Palazzo P, Pomponi D, Quaratino D, Rasi C, Zaffiro A, Zennaro D, Mari A. Clin Exp
26 Allergy. 2010 Jun;40(6):911-21. doi: 10.1111/j.1365-2222.2010.03470.x. Epub 2010 Mar 1). We
27 quoted these studies in the revised discussion and actually found that they support our data
28 regarding the molecular IgE sensitization profiles in the UK, Italy and Germany (see [lines 313-](#)
29 [317](#) of revised manuscript)-
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41 Moreover, the studies have already started up to 20 years ago and the data
42 should be available how the sensitization profiles led to allergic
43 diseases and the underlying sensitization profiles. Linking the current
44 data with the clinical datasets would improve the outcome of this study.
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49 **Reply:** We thank the reviewer for this good comment but the goal of our study was to
50 determine the molecular IgE sensitization profiles and not the longitudinal development of IgE
51 sensitizations towards clinical symptoms later on. In fact, we and others have earlier performed
52 several such analyses for cohorts with large numbers of longitudinally collected samples from
53 the same children. However, this would have been outside the scope of our current study and
54 difficult to perform because sample sizes from the individual cohorts analysed in this study
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3 were lower. Nevertheless, we have quoted our longitudinal studies in the revised discussion
4 (see [lines 348-351](#)).
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8 Detailed criticism: It is obvious and expected that different geographical
9 areas account for different aeroallergen sensitization patterns and this
10 has already been published in several publications. “We show regional,
11 exposome and climate dependent differences in molecular IgE-reactivity
12 profileswhich may form abasis for precision medicine-based
13 approaches for treatment and preventions of allergy” This statement
14 remains unclear and needs refinement. Regarding grass pollen allergens as
15 the most prevalent pollen allergens across Europe is shown. It would be
16 interesting to know if weed pollens in southern Europe are of that
17 relevance in quantity and quality as they have been described in the
18 literature.
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32 **Reply:** Following the reviewers suggestion we have refined our statement. In fact, there are
33 only a few studies which have analysed molecular sensitisation profiles but there is only one by
34 Siroux which has looked into different regions of a country and described strong differences in
35 molecular sensitization profiles (i.e., reference 14 in our paper: Specific IgE and IgG measured
36 by the MeDALL allergen-chip depend on allergen and route of exposure: The EGEA study. Siroux
37 V, Lupinek C, Resch Y, Curin M, Just J, Keil T, Kiss R, Lødrup Carlsen K, Melén E, Nadif R, Pin I,
38 Skrindo I, Vrtala S, Wickman M, Anto JM, Valenta R, Bousquet J. J Allergy Clin Immunol. 2017
39 Feb;139(2):643-654.e6. doi: 10.1016/j.jaci.2016.05.023. Epub 2016 Jun 22). In fact, it was very
40 interesting for us to see that there can be quite substantial differences between regions in
41 Spain and Italy which are not too far from each other but differ regarding climate and
42 exposome. We have refined our statement to reflect this finding better. Furthermore we
43 named precision medicine-based forms of primary prevention and treatment such as allergen-
44 specific avoidance and allergen-specific immunotherapy. In particular allergen-specific
45 immunotherapy requires the precise identification of the disease-causing allergen source which
46 can be obscured by cross-reactivity when only tested with allergen extracts. See [lines 313-320,](#)
47 [355-364](#) of the revised manuscript.
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54 The reviewer makes another very good point regarding the importance of weed pollen
55 allergens. In fact, allergy to weed pollen allergens seems to be rather limited to certain specific
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3 regions. For example, ragweed allergy is extremely common and frequent in the Lyon region in
4 France but does not play an important role in the rest of the country (see reference 14 in our
5 paper: Specific IgE and IgG measured by the MeDALL allergen-chip depend on allergen and
6 route of exposure: The EGEA study. Siroux V, Lupinek C, Resch Y, Curin M, Just J, Keil T, Kiss R,
7 Lødrup Carlsen K, Melén E, Nadif R, Pin I, Skrindo I, Vrtala S, Wickman M, Anto JM, Valenta R,
8 Bousquet J. *J Allergy Clin Immunol*. 2017 Feb;139(2):643-654.e6. doi:
9 10.1016/j.jaci.2016.05.023. Epub 2016 Jun 22). We have now mentioned in the revised results
10 IgE sensitization to certain weeds like mugwort and Parietaria as documented by Art v 1 and Par
11 j 2 sensitization, respectively to highlight their relevance in different parts of Europe. See [lines](#)
12 [220-224](#) of the revised manuscript.
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19 Regarding class 1 food allergens peanut allergens are observed
20 in certain areas – is there a good explanation why not equally
21 distributed throughout Europe? Is this really a matter of food processing?
22 What about different eating habits and life style? What about consumption
23 rates of tree nuts such as hazelnut and walnut? Different sensitization
24 patterns for HMD allergens are also provided in this study. While the
25 north south difference for sensitization rate is known from other studies
26 it is interesting to see that HMD sensitization is higher in southern dry
27 climate as compared to a southern humid area. Is there an explanation for
28 this finding?
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41 **Reply:** We thank the reviewer for the good suggestions to highlight the topic of class 1 food
42 allergens. Indeed we think that sensitisation to class 1 food allergens is related to eating habits
43 and lifestyle but also other possibilities of sensitisation, for example via the skin in case of
44 peanut may be considered. See revised manuscript [lines 397-402](#).
45
46

47 Regarding IgE sensitization there seems to be a misunderstanding. We report that IgE
48 sensitization to house dust mite allergens is more common in the warm and humid region of
49 Gipuzkoa in Spain than in the warm and dry region of Sabadell in Spain. We have clarified this in
50 the revised manuscript, see [lines 326-329](#).
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54 **Reviewer: 5**
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3 COMMENTS FOR THE AUTHOR(S)
4

5 Gea Kiewiet et al sought to investigate the molecular IgE sensitization
6 map of Europe by analysing 2800 sera from children of several cohorts from
7 different regions of Europe (North, Central/Western and South) with
8 micro-arrayed purified allergens. The authors show regional, exposome and
9 climate-dependent differences in molecular IgE-reactivity profiles within
10 the studied regions from Europe. Apart from the limitations of the study
11 indicated in the discussion section, the authors should further explain
12 and discuss the following issues:
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23 1. What is the novelty of the obtained
24 results and clinical translation/impact of the reported data/discussions?
25 The authors should clearly state what are the new findings (not in terms
26 of study design but in terms of data/conclusions) reported in this
27 manuscript.
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35 **Reply:** We thank the reviewer for allowing us to present the new finding better. In fact, as
36 pointed out in our reply to reviewer 4 we would like to repeat "It was very interesting for us to
37 see that there can be quite substantial differences in molecular IgE sensitization profiles and
38 frequencies of IgE sensitization between regions which are geographically quite close, e.g., in
39 Spain and Italy but differ regarding climate and exposome. We have pointed this out in the
40 revised discussion. Furthermore we named precision medicine-based forms of primary
41 prevention and treatment such as allergen-specific avoidance and allergen-specific
42 immunotherapy. In particular allergen-specific immunotherapy requires the precise
43 identification of the disease-causing allergen source which can be obscured by cross-reactivity
44 when only tested with allergen extracts. See [lines 313-320, 355-364](#) of the revised manuscript."
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51 2. The selected regions seem rather arbitrary and not
52 necessarily resembling the heterogeneity reported in such European
53 regions. The included areas do not fully represent such heterogeneity.
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3 This should be discussed in more detail.
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7 **Reply:** We agree with the reviewer. In fact, it is one limitation of our study that we have
8 analysed only sera from regions where birth cohorts had been established. We think that it will
9 be important to continue to study the investigation of molecular IgE sensitization profiles by
10 conducting cross-sectional studies analysing sera from subjects who were born and grew up in
11 different regions of different European countries to obtain a high resolution picture. This was
12 mentioned in the revised discussion [lines 445-449](#).
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18 3. This is a retrospective cross-sectional study including sera from children
19 aged 1-16 years. As this is not a prospective follow-up study, some of the reported
20 conclusions in terms of onset and development of IgE sensitization
21 profiles should be ameliorated.
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28 **Reply:** Following the reviewers recommendation we ameliorated conclusion regarding onset
29 and development of IgE sensitization profiles, see [lines 1-3, 119, 125, 187-194, 212, 346-351,](#)
30 [459-460](#) of revised manuscript.
31
32
33

34 4. Is UK regiotype comparable to Central/Western Europe countries (i.e. Germany)?
35

36 **Reply:** We thank the reviewer for this good question. Despite the fact that sera obtained in the
37 BiB cohort were from young children (age 4) and whereas German children were older (i.e., 15-
38 16 years) one can say that the profile of recognized respiratory allergens was similar with the
39 exception of house dust mite allergens which were more frequently recognized in the UK
40 cohort. Regarding class 1 food allergens it seems that sensitization to peanut allergen molecules
41 and to the major fish allergen Gad c 1 is highly frequent in the UK children but not in the
42 children from Germany. We mentioned this interesting findings in the revised discussion (see
43 [lines 400-402](#) of revised manuscript).
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47 5. The discussion is too long. It should be shortened to avoid redundancy of data already
48 presented in the result section.
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52 **Reply:** Following the reviewers suggestion we shortened the discussion by removing repetitions
53 of results, see [lines 300-313](#) of revised manuscript.
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Rudolf Valenta

For Peer Review

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3 **1 A molecular sensitization map of European children reveals exposome- and**
4 **2 climate-dependent sensitization profiles**
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8 4 M. B. Gea Kiewiet^{1#}, Christian Lupinek^{2##}, Susanne Vrtala², Sandra Wieser²⁺, Alexandra Baar², Renata
9 Kiss², Inger Kull^{3,4}, Eric Melén^{3,4,5}, Magnus Wickman^{3,4}, Kai-Hakon Carlsen⁶, Karin Lodrup-Carlsen⁶,
10 Daniela Porta⁷, Davide Gori⁸, Ulrike Gehring⁹, Rob Aalberse¹⁰, Jordi Sunyer¹¹, Marie Standl¹², Joachim
11 Heinrich¹², Dagmar Waiblinger¹³, John Wright¹³, Josep M. Antó¹⁴, Jean Bousquet^{15, 16, 17, 18}, Marianne
12 van Hage^{1*}, Rudolf Valenta^{2,19,20,21*}
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24 47

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35 54

36
37 55 **Running title:** Molecular sensitization map of Europe

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39 56

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41
42
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44 59 assistance regarding manufacturing of the customized allergen arrays which were made at Phadia
45 60 Austria GmbH, Part of Thermo Fisher Scientific ImmunoDiagnostics, A-1220, Vienna, Austria. This
46 61 paper is dedicated to Prof. Jean Bousquet for his amazing leadership in the MeDALL project.

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48
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51 64 Council (ALF project), The Swedish Asthma and Allergy Association's Research Foundation, The
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54 67 European Commission, by Mead Johnson, Evansville, Indiana, USA, by Nestle, Vevey, Vaud,

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For Peer Review

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3 74 **Abstract**

4
5 75 **Background:** Understanding differences in sensitization profiles at the molecular allergen level is
6
7 76 important for diagnosis, personalised treatment and prevention strategies in allergy.
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10 78 **Methods:** IgE sensitization profiles were determined in more than 2800 sera from children in 9
11 79 population-based cohorts in different geographical regions of Europe; north (BAMSE (Sweden), ECA
12 80 (Norway)), west/central (PIAMA (the Netherlands), BiB (UK), GINIplus (Germany)), and south (INMA
13 81 Sabadell and Gipuzkoa (Spain) and ROBBIC Rome and Bologna (Italy)) using the MeDALL-allergen
14
15 82 chip.
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19 84 **Results:** Sensitization to grass pollen allergen, Phl p 1, and to major cat allergen, Fel d 1, dominated
20 85 in most European regions whereas sensitization to house dust mite allergens Der p 1, 2 and 23 varied
21 86 considerably between regions and were lowest in the north. Less than half of children from Sabadell
22 87 which has a hot and dry climate were sensitized to respiratory allergens, in particular house dust
23 88 mite allergens as compared to Gipuzkoa nearby with a more humid climate. Peanut allergen Ara h 1
24 89 was the most frequently recognized class 1 food allergen in Northern/Western Europe, while the
25 90 fruit allergens Pru p 3, Act d 1 and 2 were prominent in Southern and Western/Central Europe. Ves v
26 91 5-sensitization dominated in North and West/Central Europe.
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35 93 **Conclusion:** We show regional, exposome and climate-dependent differences in molecular IgE-
36 94 reactivity profiles in Northern, Western/Central and Southern Europe which may form a molecular
37 95 basis for precision medicine-based approaches for treatment and prevention of allergy.
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45 98 **Keywords:** Allergen molecules, IgE-reactivity, Europe, exposome, MeDALL chip, sensitization profile
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101 Introduction

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

108 In 2010, the European Union-funded project "MeDALL" (Mechanisms of the development of
109 allergies) was initiated, a framework for research institutions specialized on various "omics"-
110 technologies to join forces with groups conducting birth cohorts
111 (<https://cordis.europa.eu/project/rcn/96850/factsheet/en>). This gave us the unique opportunity to
112 compare the molecular IgE sensitization profiles from 9 different population-based cohorts located in
113 different geographical regions of Europe; Northern (BAMSE⁵ (Sweden), ECA⁶ (Norway)), West/Central
114 (PIAMA⁷ (the Netherlands), BiB⁸(UK), GINIplus⁹(Germany)), and Southern (INMA¹⁰ Sabadell and
115 Giupuzcoa (Spain) and ROBBIC¹¹ Rome and Bologna (Italy)) Europe. Together these cohorts
116 comprised sera from more than 2800 children between the age of 1 to 16 years, allowing to compare
117 also to some extent the evolution of sensitizations from early childhood to adolescence in the
118 different regions of Europe. For this comprehensive IgE-testing, a customized allergen microarray,
119 the MeDALL-chip, was developed that covered 176 allergens and proved superior regarding
120 sensitivity and coverage of allergen molecules as compared to available diagnostic tests.^{12,13} The
121 results of our analysis provide for the first time a comprehensive, high-resolution atlas of IgE-
122 sensitization rates and patterns from the general population from different regions of Northern,
123 Western/Central and Southern Europe.

125 **Materials and methods**

126

127 **Cohorts and design of the study**

128 IgE measurements were performed retrospectively on sera from 2855 children, aged 1-16 years,
129 from nine different birth cohorts representing the northern, west/central and southern part of
130 Europe. Two cohorts from Northern Europe, BAMSE⁵ (Sweden) and ECA⁶ (Norway), 3 cohorts from
131 Western/Central Europe, PIAMA⁷ (The Netherlands), BiB⁸ (UK), and GINIplus⁹ (Germany), as well as
132 four cohorts from Southern Europe, INMA¹⁰ (Spain, Guipuzcoa and Sabadell) and ROBBIC¹¹ (Italy,
133 Bologna and Rome) were included and information regarding the cohorts can be found in references
134 ⁵⁻¹¹. (Figure 1). For individual cohorts, blood collection had been scheduled for different ages. This
135 allowed us to some extent to also investigate IgE sensitization between children of 1, 4, 7-12 and 15-
136 16 years of age. The exact location, participant age and numbers of analyzed sera of each cohort are
137 summarized in Table 1. Sera were randomly picked within each cohort taking into consideration that
138 only sera from children who were born in the region and spent at least the first year of life there
139 were analyzed. Furthermore, we aimed at a gender balance regarding the samples. In those cohorts
140 where different time points were studied sera were taken from children for whom samples were
141 available at each of the time points of sampling. For each of the cohorts ethics approval and written
142 informed consent from the parents or legal guardians of the children was available for the analysis of
143 allergen-specific IgE⁵⁻¹². The analysis of pseudonymised serum samples was performed at the
144 Department of Pathophysiology and Allergy Research, Medical University of Vienna, Austria in a
145 centralized manner with permission of the Ethics committee of the Medical University of Vienna,
146 EK1641/2014. Possible limitations of the study are mentioned in the discussion section.
147 (<https://www.strobe-statement.org/>).

148

149 **MeDALL-chips**

150 The customized MeDALL-chips were obtained from Phadia Austria GmbH, Part of Thermo Fisher
151 Scientific ImmunoDiagnostics, A-1220, Vienna, Austria. Allergen microarrays were prepared
152 according to the ImmunoCAP ISAC technology with some slight modifications and had been
153 compared with traditional forms of allergy diagnosis in earlier studies^{12, 13}. More detailed information
154 can be found in the supplementary information about quality controls and subsequent measures
155 (Tables E1-E2).

156

157 **Data analysis**

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3 158 All analyses were performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk,
4
5 159 NY, USA). First, allergen molecules were grouped according to their exposure route. We identified a
6
7 160 group of respiratory allergens, food allergens and 'other' allergens, which induce sensitization via
8
9 161 different routes, including insect and latex allergens. Cross-reactive Carbohydrate Determinants
10
11 162 (CCD)-bearing allergen molecules were analyzed as a separate group. Please note that the terms CCD
12
13 163 marker and MUXF3 (i.e., Ana c 2.0101) are used in a synonymous manner throughout the manuscript
14
15 164 and differ from the term "CCD-bearing allergen" which designate protein allergens containing
16
17 165 protein-bound CCDs. For each cohort and age group, allergic sensitization rates (percentage of IgE-
18
19 166 positive subjects) were calculated for each allergen. All allergen molecules were ranked based on the
20
21 167 sensitization frequencies and listed by group (Tables E3-E6). The median (minimum-maximum) ISU
22
23 168 levels were also provided. From these tables, the 10 highest ranked primary (i.e., non-cross-reactive)
24
25 169 allergens were extracted for each cohort and age (Figures 2-5). For these allergen molecules, the
26
27 170 percentage of subjects with IgE levels were grouped according to ISU class ranges (low = ≥ 0.3 -1 ISU,
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29 moderate = 1-15 ISU, high > 15 ISU).
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173 Results

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175 **Frequencies of detectable molecular IgE sensitization to respiratory allergens and class I** 176 **food allergens vary in the cohorts and increase by age but without major qualitative** 177 **alterations within the cohorts with follow-up samples**

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

187

188 **Grass pollen allergens are the major pollen allergens in almost all European regions**

189 The top-10 primary respiratory allergen molecules ranked by sensitization rate are shown in Figure 2.
190 Timothy grass allergens were prominent in all cohorts from the age of 4, except in INMA Sabadell. At
191 the age of 7-12 years, children in all cohorts were sensitized to the allergens Phl p 1, 5b, 6, 2, 11 and
192 12 (Table E3). Phl p 1 was the dominant allergen throughout all the cohorts. However, frequencies of
193 IgE sensitization to Phl p 1 were highest in PIAMA, followed by BAMSE and ECA, and were lowest in
194 the southern cohort ROBBIC. Phl p 7 was recognized in northern and western/central cohorts, but
195 not in southern ones.

196

197 **Sensitization to tree and weed pollen allergens in the different European regions reflects** 198 **the quality of allergen exposure, the exposome**

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

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2
3 206 In contrast, olive allergen Ole e 1-specific IgE was mainly detected in the southern cohorts.
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5 207 Both at the age of 4 and 7-12 years, Ole e 1 sensitization was higher in INMA and ROBBIC
6
7 208 respectively, compared to all other cohorts. In addition, the cypress allergen Cup a 1 was prominent
8
9 209 in the ROBBIC Rome cohort (Figure 2, Figure 5).

10 210 Regarding weed pollen allergens we found that the major mugwort allergen, Art v 1 was
11
12 211 quite frequently recognized by children from the BAMSE and ECA cohort (Table E3, Figure 2) and the
13
14 212 major Parietaria allergen, Par j 2, showed frequent IgE reactivity in children from the ROBBIC cohort
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16 213 in Rome which fits to the vegetation profiles in these areas. Interestingly, the major ragweed
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18 214 allergen, Amb a 1, did not seem to be relevant in the cohorts tested by us.

19 215

20 216 **Fel d 1 is an important indoor allergen in almost all European regions whereas frequencies** 21 217 **of sensitization to house dust mite allergens vary considerably**

22 218 The cat allergen Fel d 1 was the most frequently recognized pet allergen molecules in all cohorts and
23
24 219 ages except for INMA Guipuzcoa. At 4 years, the sensitization frequency to Fel d 1 was highest in
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26 220 BAMSE (8.7%), BiB (7.2%) and PIAMA (5.6%), whereas it was low in INMA Sabadell (1%) and INMA
27
28 221 Guipuzcoa (0.5%) (Figure 2). Around 20% of the oldest children (age 15-16 years) were sensitized to
29
30 222 Fel d 1 in GINI, ECA and BAMSE (Figure 2, Table E3). Sensitization to house dust mite allergens varied
31
32 223 considerably in the different regions. For the western/central and southern cohorts, the house dust
33
34 224 mite allergens Der p 1, 2 and interestingly also Der p 23 were among the allergen molecules with the
35
36 225 highest recognition frequencies (Figure 2). Also, Der p 5, 7, 15, and 37 were often recognized (Table
37
38 226 E3). However, house dust mite allergens were only minor allergens in the northern cohort BAMSE at
39
40 227 all ages. However, we also noted striking differences regarding sensitization to house dust mites in
41
42 228 Southern Europe. In Sabadell which is close to Guipuzcoa in Spain, less than half of the children were
43
44 229 sensitized to house dust mite allergens (Figure 2). In addition, the fungus allergen Alt a 1 was
45
46 230 prominent only in the southern cohorts. It was the most frequently recognized respiratory allergen in
47
48 231 INMA Sabadell at 4 years and the second most recognized component in ROBBIC Bologna at 7-12
49
50 232 years (Figure 2).

51 233

52 234 **Genuine sensitization to peanut allergens is frequent only in certain regions**

53 235 The top-10 class 1 food allergen molecules ranked by sensitization rate are shown in Figure 3. The
54
55 236 major peanut allergen Ara h 1 was the most frequently recognized class 1 food allergen in the BiB
56
57 237 cohort (6.8%) and the second most recognized in the BAMSE cohort (4.9%) at 4 years. In all other
58
59 238 cohorts at this age the sensitization rate was very low. At older ages Ara h 1 was the most recognized
60
239 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

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3 273 (Cypress) was found to be prominent in the ROBBIC Rome cohort (15.7%) with approximately the
4 double percentage compared to Phl p 4, while sensitization frequencies were low in all other cohorts.
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6 275 A frequently recognized CCD-containing food allergen was the walnut allergen Jug r 2. It was
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8 276 ~~recognized~~ detected in all cohorts and age groups, mostly in relatively low frequencies ($\leq 4.5\%$).
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10 277 Interestingly, sensitization rates to the pure CCD marker MUXF3 were similar in all cohorts and rather
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12 278 low (i.e., approximately 1-2%) (Table E6).
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280 Discussion

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

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

290 Striking regional differences regarding molecular IgE sensitization profiles in the different
291 cohorts were observed. There are only few previous studies which have analyzed molecular IgE
292 sensitization profiles in population-based cohorts from individual countries (e.g., UK, Germany, Italy)
293 which in fact confirm the molecular sensitization patterns which we observed for these countries^{14, 15,}
294 ¹⁶. However, there is only one study which involved different regions of France and demonstrated
295 that there can be important differences regarding molecular sensitization profiles based on
296 differences in the regional exposome. In fact, Siroux et al showed that the sensitization profile of
297 people from five different regions even within one country (i.e., France) differed significantly, which
298 was reflected in the differences in vegetation between the studied areas.¹⁷ Also in our study the
299 exposome and in particular the climate seemed to show local differences as observed between the
300 cities of Rome and Bologna as well as Sabadell and Gipuzkoa in Italy and in Spain, respectively.

301 The fact that the climate in Sabadell is much more hot and dry¹⁸ than in Gipuzkoa¹⁹ may be a
302 reason why less than half of the children of the same age (i.e., 4 years) were sensitized to respiratory
303 allergens, in particular to house dust mite allergens. Thus frequencies of IgE sensitization to
304 respiratory allergens in children at four years were especially low in the dry and hot region of
305 Sabadell as compared to cohorts from North-, West- and Middle Europe.

306 When scrutinizing differences between the cohorts, we first observed that Phl p 1, the major
307 timothy grass pollen allergen, was the dominant allergen in all investigated regions due to the
308 ubiquitous distribution of grasses. Phl p 1 has been suggested to initiate the sensitization process to
309 timothy grass in pollen allergic children.^{20, 21} Furthermore, Phl p 1 is highly cross-reactive with group 1
310 allergens in different grass species and unlike other grass pollen allergen groups, group 1 allergens
311 occur in all grass species²², which is reflected in the high frequency of sensitization against grasses in
312 general in all regions. However, sensitization against Phl p 1 and other timothy grass allergens were

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3 313 not detected in subjects of the INMA Sabadell cohort. Again, this it likely due to the dry and hot
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5 314 climate there.²³

6 315 For tree pollen allergens significant differences in sensitization profiles were found between
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8 316 Northern/Central and Southern Europe, which clearly reflect the different tree exposomes in these
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10 317 regions. Birch trees are most common in Northern and Central Europe.^{24, 25} In line with this, Bet v 1,
11 318 the major birch allergen, was already prominent in the BAMSE and ECA cohorts (Northern Europe) at
12 319 a young age, while it played a more significant role in PIAMA and GINI (Western/Central Europe) in
13 320 older children suggesting an increase of detectable IgE sensitization by age. However, for most of the
14 321 cohorts we did not have follow up samples to draw firm conclusions regarding the longitudinal
15 322 development of IgE sensitizations and the associated development of symptoms. Such studies have
16 323 been performed so far only for certain allergen sources and in certain cohorts²⁶⁻³⁰ and were not the
17 324 topic of our study which aimed to provide a comprehensive picture of molecular IgE sensitizations in
18 325 different regions of Europe.

19 326 In contrast to Northern Europe, Italy and Spain, where olive trees are responsible for a significantly
20 327 part of airborne pollens³¹, sensitization was observed in the INMA and ROBBIC cohorts. Cypress is
21 328 another typical Mediterranean tree found above all in Italy³² which was reflected in the dominance
22 329 of Cup a 1 sensitization mainly in Rome. Like for Gipuzkoa and Sabadell, two close regions in Spain we
23 330 noted strong differences regarding molecular IgE sensitization profiles between Bologna and Rome.
24 331 In Bologna sensitizations to grass pollen allergens dominated whereas in Rome sensitization to HDM
25 332 allergens were more frequent. We think that it is an important finding of our study that we detected
26 333 strongly varying molecular IgE sensitization profiles even in regions which are close to each other
27 334 within one country because this finding has important implications for precision medicine
28 335 approaches such as allergen avoidance (e.g., HDM allergy) and accurate prescription of allergen-
29 336 specific immunotherapy. Molecular diagnosis is especially important for the precise identification of
30 337 the genuinely sensitizing allergen sources which can be obscured by cross-reactivity when allergen
31 338 extracts are used.

32 339 The major cat allergen Fel d 1 was the most frequently recognized allergen among the furry
33 340 animals. When comparing the European regions, Fel d 1 sensitization was most common in Northern
34 341 and Central Europe already from a young age, while sensitization frequencies were lower in Southern
35 342 Europe. A similar profile was recently also described for the Moscow region of Russia, where Fel d 1
36 343 was the most frequently recognized indoor allergen.³³ The data is in line with a report showing that a
37 344 higher percentage of people in Norway, Sweden and the UK had a cat during childhood.³⁴ However,
38 345 since multiple factors have been found to affect Fel d 1 levels, including keeping cats indoors,
39 346 smoking habits and ventilation, it still remains unclear why Fel d 1 levels in house dust are lower in

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3 347 southern Europe.³⁵ One possibility though may be that cats are less often kept indoor in these
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5 348 countries due to the climate.

6 349 The presence of house dust mite allergens, both Der p and Der f, depends on humidity.^{36, 37}
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8 350 This is in line with our finding that IgE to the house dust mite allergens were almost absent in BAMSE,
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10 351 very low in Sabadell, present in ECA and PIAMA and most prominent in the BiB cohort from the UK.
11 352 In most cohorts sensitization was observed against several of the major house dust mite allergens,
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13 353 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
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15 354 Unlike house dust mites, the fungus *Alternaria alternata*, has shown to be an indoor allergen which
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17 355 grows better in a dry and warm climate.²² As a result, Alt a 1 was found to be one of the most
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19 356 important allergens only in the cohorts from Sabadell (Spain) and Bologna (Italy).

20 357 Regarding food allergens our study differs from the EuroPrevall study which has focused on
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22 358 food-allergic subjects and only few molecular analyses focusing on certain food allergens have been
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24 359 performed within EuroPrevall³⁹. By contrast, our study has investigated random population samples
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26 360 from different parts of Europe for IgE sensitizations to food allergens. We found that Ara h 1 and 2
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28 361 are clearly the most prominent allergens in BAMSE and BiB, but rare in the other cohorts.
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30 362 Geographical differences in clinical and immunological profiles of peanut allergens have been
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32 363 reported. Vereda et al. showed that peanut allergic patients from the US and Sweden recognized the
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34 364 storage proteins Ara h 1-3 more frequently compared to Spanish patients who were more often
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36 365 sensitized against the lipid transfer protein Ara h 9.⁴⁰ We also noted that Ara h 9 sensitization was
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38 366 higher in the southern cohorts INMA Gipuzkoa and ROBBIC Rome compared to BAMSE. These
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40 367 differences are not only depending on the amount and timing of peanut consumption. A study from
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42 368 Sweden has shown that the increase in peanut sensitization over the years is not only due to
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44 369 increased peanut consumption.⁴¹ Differences in preparation of peanuts also plays a role. Roasted
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46 370 peanuts, which are consumed more in Sweden, the US and other western countries, contain more
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48 371 stable proteins and thus may have a higher allergenicity.⁴² Regarding peanut differences of allergen
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50 372 contact via the skin may also be considered to be responsible for different sensitization rates in the
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52 373 different populations besides nutritional habits.⁴³ High sensitization rates to peanut allergens and to
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54 374 the major fish allergen Gad c 1 in the BiB cohort from UK as compared to other cohorts may be an
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56 375 example for such nutritional habits. However, sensitization against the dominant shrimp allergen Pen
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58 376 m 1 may reflect to some extent cross-reactivity with the tropomyosin Der p 10. In individual patients,
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60 377 specific IgE levels to Pen m 1 and Der p 10 and IgE cross-inhibition studies may inform which allergen
378 may have been the genuinely sensitizing molecule. Act d 1 sensitization was found to be prominent
379 only in southern Europe, where kiwifruit is grown locally, and especially Italy is known for its high
380 kiwifruit consumption.⁴⁴

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3 381 Regarding venom allergens, our study provides new and unexpected information, since data
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5 382 on hymenoptera IgE sensitization are scarce, especially in children. We found that between 7 and
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7 383 20% of 15-16 year olds from the northern cohorts showed IgE-reactivity against the major wasp
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9 384 venom Ves v 5 while a considerably lower rate of sensitization was found at younger ages, which is in
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11 385 line with data reported previously.⁴⁵ Although we did not have data from Southern Europe for the
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13 386 15-16 year olds, Ves v 5 sensitization seems to be less frequent in this area at a younger age. The
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15 387 most important wasp species, belonging to the *Vespula* genus and responsible for Ves v 5
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17 388 sensitization, have been found to be present all over Europe, but more precise data on their
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19 389 geographical distribution and population density are lacking, which makes it difficult to explain the
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21 390 observed differences in sensitization frequency.⁴⁶ We speculate that children in Northern Europe
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23 391 could be more exposed to wasps, for example because they spend more time outdoors and in nature
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25 392 during the summer period.

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27 393 With respect to IgE-positivity to natural allergen molecules bearing cross-reactive
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29 394 carbohydrate determinants (CCDs), similar rates were observed throughout all regions of Europe,
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31 395 with Phl p 4 being the most prominent CCD-bearing allergen. For these CCD-bearing allergen
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33 396 molecules, coming mainly from plants, it is impossible to distinguish IgE-reactivity to the sugar
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35 397 moieties from antibody-binding to the protein backbone at an individual level. However, only in
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37 398 southern cohorts IgE-levels to nCup a 1 were found to be indicative for true sensitization to those
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39 399 trees (cypress, cedar) or grasses (Bermuda grass) that are native in those regions. The remarkably
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41 400 high prevalence of IgE-positivity to nJug r 2 in the German GINI-cohort can presumably be partly
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43 401 attributed to reactivity with CCDs present on this glycoprotein, while in other cohorts reactivity to
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45 402 nJug r 2 was paralleled by an increase of IgE to Jug r 1, indicative of genuine IgE-sensitization to
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47 403 walnut. Regarding the only CCD marker (i.e., MUXF3) which was tested in each of the cohorts a
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49 404 relatively low frequency (approximately 1-2%) of IgE reactivity was found indicating that for CCD-
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51 405 bearing allergens also protein IgE epitopes play a role.

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53 406 It is one limitation of our study that not all children from whom sera had been collected had
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55 407 exactly the same age but this should not affect the major findings of the study which are that
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57 408 sensitization profiles to allergen molecules seemed to vary regarding the allergen exposome and
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59 409 climate in the different cohorts and remained largely unaltered over time. Another limitation of our
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410 study is that we have not taken into account the atopic background of the parents of children when
411 picking the serum samples from children but it seems that the atopic background of parents does not
412 have such strong effects on allergic sensitization in children⁴⁷. Likewise, we have not stratified
413 children according to genetic background, ethnicity, nutritional habits and environmental pollution.
414 However, in a recent study we did not find much evidence that pollution would influence allergen-

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3 415 specific IgE sensitization⁴⁸. Other limitations of our study are that we make only descriptive
4 416 comparisons without any adjustments and that the analyses were done only for available samples for
5 417 arbitrarily selected cohorts. On the other hand one may consider the arbitrary analysis of children
6 418 who were born and grew up in a region as a strength because it may provide real-life pictures of the
7 419 local molecular sensitization profiles. Furthermore, to the best of our knowledge, our study revealing
8 420 molecular sensitization profiles in a population-based cohorts of children from a continent
9 421 represents the first of its kind in the world. A more detailed molecular IgE sensitization map of
10 422 Europe and other continents may be obtained in the future by cross-sectional analyses of random
11 423 populations of patients who are recruited by questionnaires from several different regions of the
12 424 individual countries with different climate and living habits. Like in our study the patients should
13 425 have been born and grown up in the regions of investigation to inform about the influence of the
14 426 exposome and climate conditions on allergic sensitization.

15 427 In conclusion, this comprehensive data-set of high-resolution IgE-sensitization patterns of
16 428 several thousand children from population-based European birth cohorts, with a north, south and
17 429 west/central gradient, provides a detailed overview of regional differences in IgE-reactivity profiles of
18 430 the general populations, which depend largely on the local exposome and climate. Since the method
19 431 used for IgE-detection was based on a commercially available platform (ImmunoCAP ISAC), our data
20 432 can be combined with existing and future data-sets from further cohorts based on this technology.
21 433 Furthermore, our sensitization map of Europe may form a basis for molecular strategies for
22 434 prevention and therapy of allergy.

23 435

24 436 **Authors contribution:**

25 437 CL, JA, JB and RV designed the study. CL and RK performed the experiments. CL and GK analyzed and
26 438 interpreted the data. IK, EM, MW, K-HC, K L-C, DP, DG, HAS, RB, UG, MS, JH, DW, JW, SV, SW, AB
27 439 collected patients' material and/or prepared and characterized allergen molecules. CL, GK, MvH, RV
28 440 contributed to data interpretation and wrote the first draft of the manuscript. All authors critically
29 441 reviewed the manuscript and approved the submitted version.

30 442

31 443 **Conflicts of interest:** R.V. receives research grants from HVD Biotech, Vienna, Austria and Worg
32 444 Pharmaceuticals, Hangzhou, China. He serves as consultant for Worg and Viravaxx AG, Vienna,
33 445 Austria. MvH has received lecture fee from Thermo Fisher Scientific. GK has no conflict of interest to
34 446 declare. CL and SW are currently employees of MacroArray Diagnostics GmbH, Vienna, Austria. JB
35 447 reports personal fees from Cipla, Menarini, Mylan, Novartis, Purina, Sanofi-Aventis, Teva, Uriach. He

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

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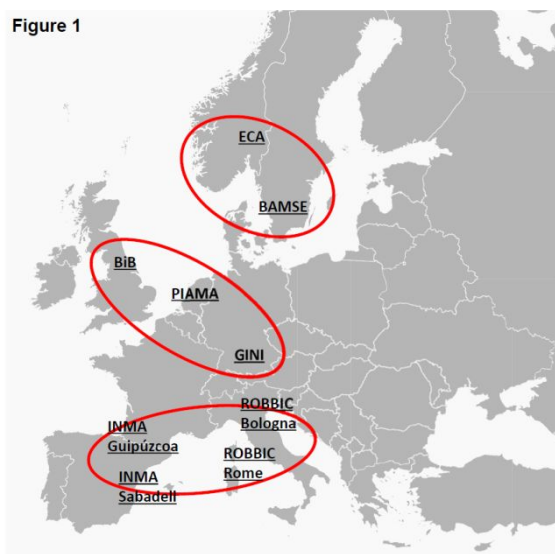
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40 511 [b-d&ei=iYQcY_rMFcKMxc8Pla-v](https://www.google.com/search?q=gipuzkoa%2C+spain+and+weather+averages&client=firefox-b-d&ei=iYQcY_rMFcKMxc8Pla-vAk&ved=0ahUKewi6teOqnor6AhVCRvEDHZXC58Q4dUDCA0&uact=5&oq=gipuzkoa%2C+spain+and+weather+averages&gs_lcp=Cgdnd3Mtd2l6EAMyBQgAEKIEOgolABBHENYEELADogclAB AeEKIESgQIQRgASgQIRhgAUPYPWNUlAWDqmAfoAnABeACAAV2IAaAJkgECMTaYAQCgAQHIAQLAAQE&sclient=gws-wiz)
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42 513 [n+and+weather+averages&gs_lcp=Cgdnd3Mtd2l6EAMyBQgAEKIEOgolABBHENYEELADogclAB](https://www.google.com/search?q=gipuzkoa%2C+spain+and+weather+averages&client=firefox-b-d&ei=iYQcY_rMFcKMxc8Pla-vAk&ved=0ahUKewi6teOqnor6AhVCRvEDHZXC58Q4dUDCA0&uact=5&oq=gipuzkoa%2C+spain+and+weather+averages&gs_lcp=Cgdnd3Mtd2l6EAMyBQgAEKIEOgolABBHENYEELADogclAB AeEKIESgQIQRgASgQIRhgAUPYPWNUlAWDqmAfoAnABeACAAV2IAaAJkgECMTaYAQCgAQHIAQLAAQE&sclient=gws-wiz)
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615 **Figures and legends**

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

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Table I

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

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

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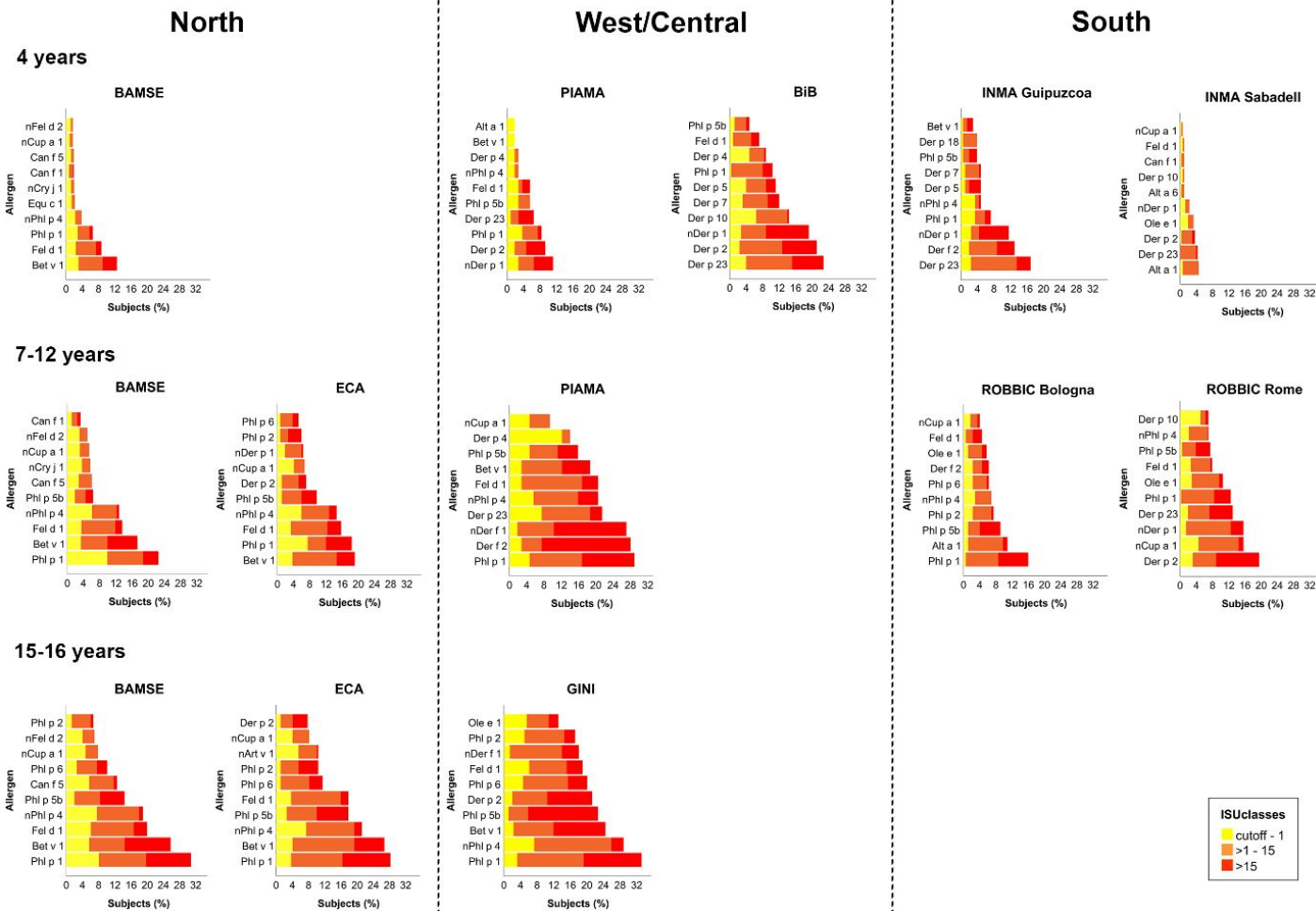
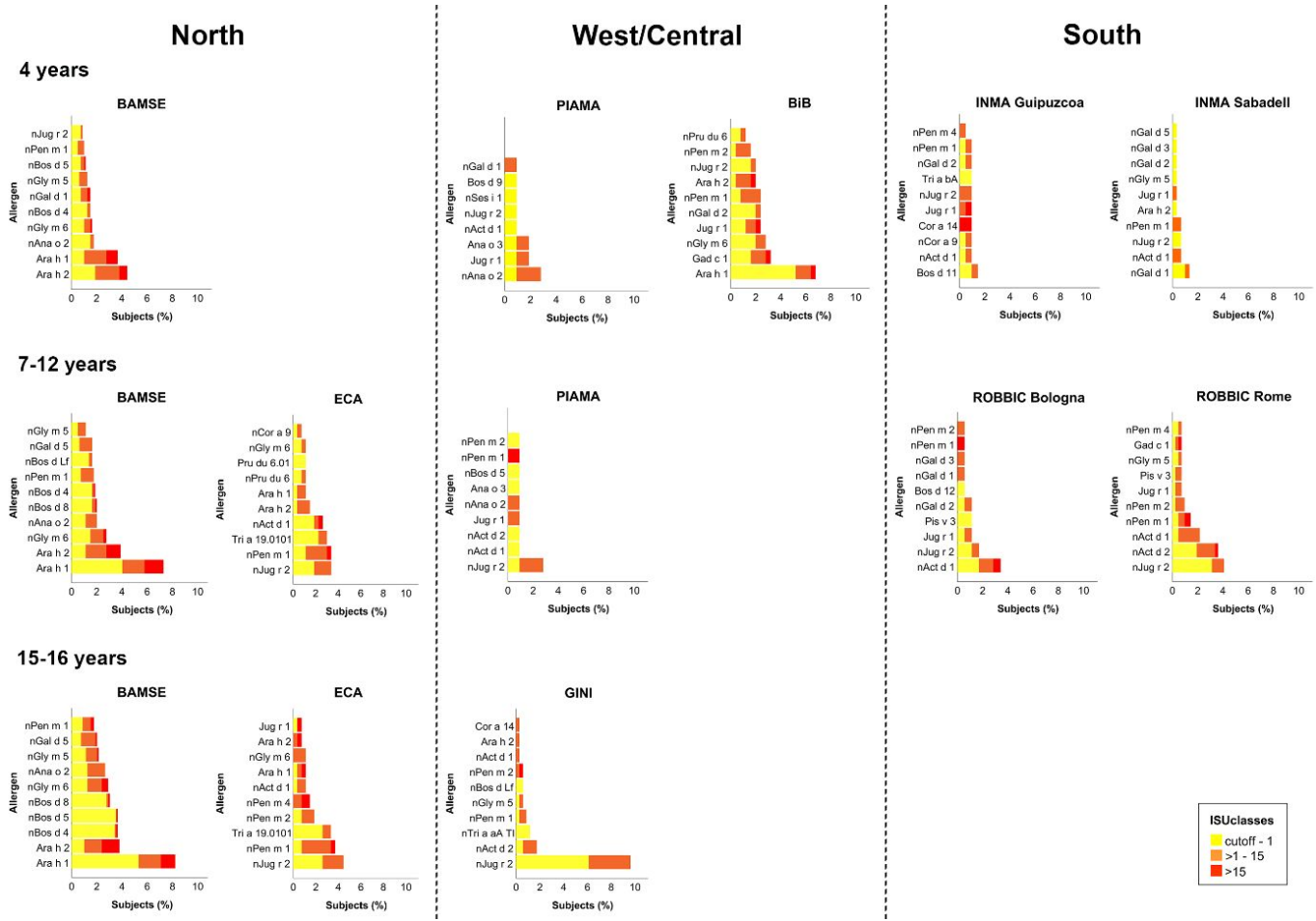


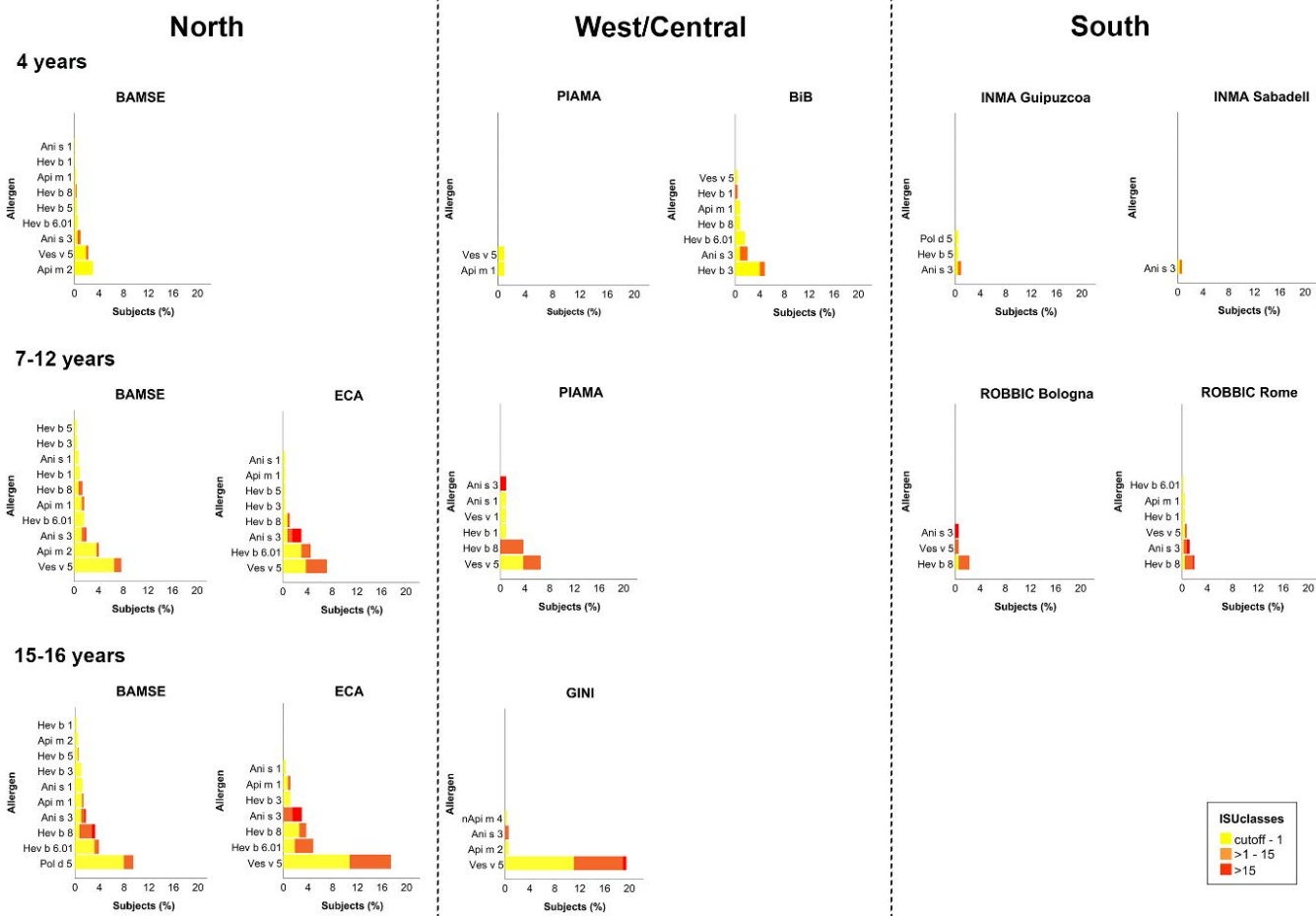
Figure 2. Overview of the 10 most frequently recognized primary respiratory allergens per cohort. The cohorts are organized based on age and region. For each cohort, allergens are ranked based on sensitization rate. Each bar shows the percentage of subjects with IgE levels within the different ISU classes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

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35 630 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,
 36 631 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 =
 37 632 1-15 ISU, red > 15 ISU).

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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).

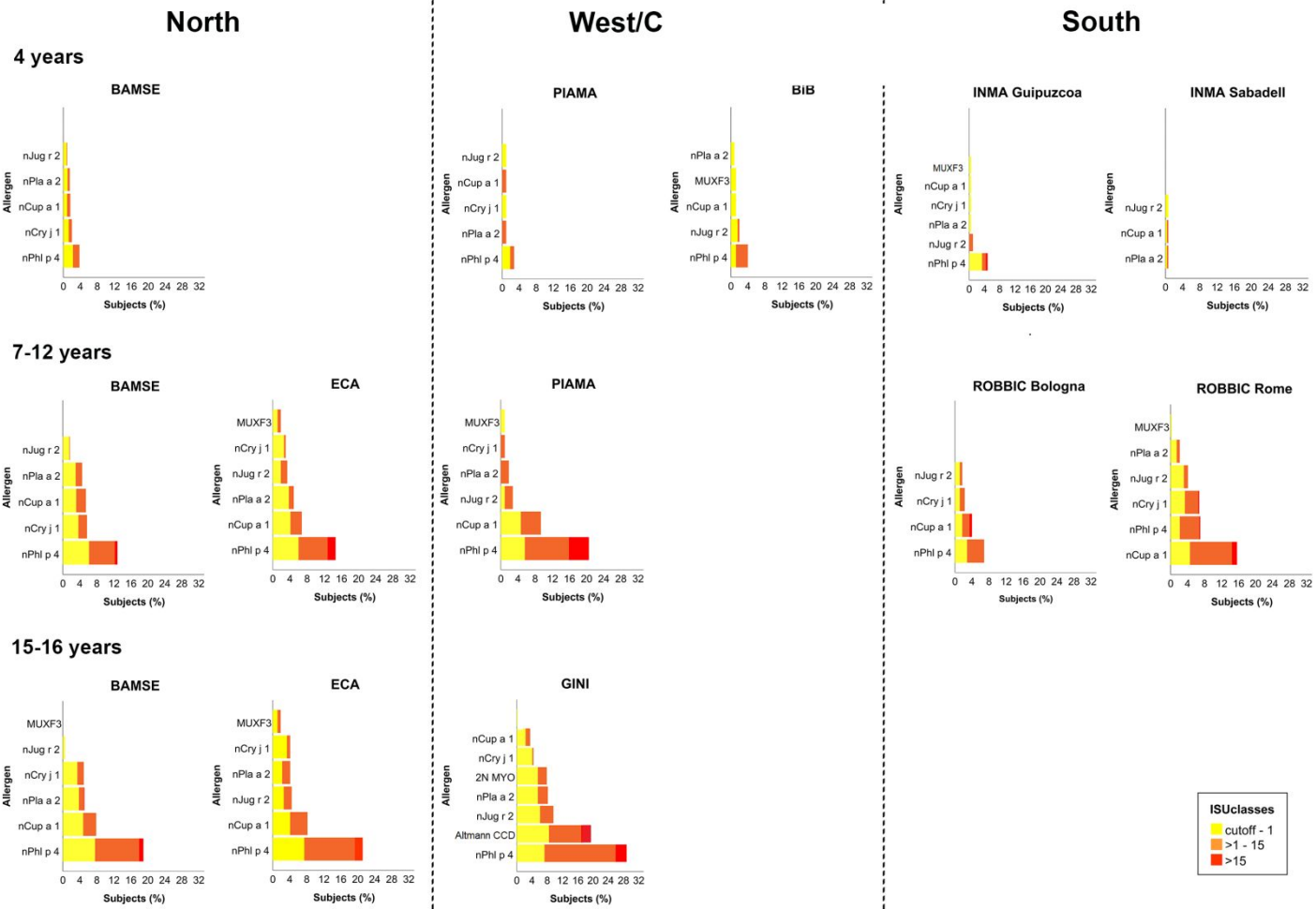


Figure 5. Overview of the most frequently recognized CCD-bearing allergens per cohort. The cohorts are organized based on age and region. For each cohort, allergens are ranked based on sensitization rate. Each bar shows the percentage of subjects with IgE levels within the ISU classes (yellow = ≥ 0.3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

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4 1 **A molecular sensitization map of European children ~~followed from childhood~~**
5 2 **~~to adolescence~~ reveals exposome- and climate-dependent sensitization**
6 3 **profiles**
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10 5 M. B. Gea Kiewiet^{1#}, Christian Lupinek^{2##}, Susanne Vrtala², Sandra Wieser²⁺, Alexandra Baar², Renata
11 6 Kiss², Inger Kull^{3,4}, Eric Melén^{3,4,5}, Magnus Wickman^{3,4}, Kai-Hakon Carlsen⁶, Karin Lodrup-Carlsen⁶,
12 7 Daniela Porta⁷, Davide Gori⁸, Ulrike Gehring⁹, Rob Aalberse¹⁰, Jordi Sunyer¹¹, Marie Standl¹², Joachim
13 8 Heinrich¹², Dagmar Waiblinger¹³, John Wright¹³, Josep M. Antó¹⁴, Jean Bousquet^{15, 16, 17, 18}, Marianne
14 9 van Hage^{1*}, Rudolf Valenta^{2,19,20,21*}

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23 55

24 56 **Running title:** Molecular sensitization map of Europe

25 57

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For Peer Review

1
2
3 75 **Abstract**

4 76 **Background:** Understanding differences in sensitization profiles at the molecular allergen level is
5 77 important for diagnosis, **personalised** treatment and prevention strategies in allergy.
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10 79 **Methods:** IgE sensitization profiles were determined in more than 2800 sera from children in 9
11 80 population-based cohorts in different geographical regions of Europe; north (BAMSE (Sweden), ECA
12 81 (Norway)), west/central (PIAMA (the Netherlands), BiB (UK), GINIplus (Germany)), and south (INMA
13 82 Sabadell and Gipuzkoa (Spain) and ROBBIC Rome and Bologna (Italy)) using the MeDALL-allergen
14 83 chip.
15
16 84

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18 85 **Results:** Sensitization to grass pollen allergen, Phl p 1, and to major cat allergen, Fel d 1, dominated
19 86 in most European regions whereas sensitization to house dust mite allergens Der p 1, 2 and 23 varied
20 87 considerably between regions and were lowest in the north. Less than half of children from Sabadell
21 88 which has a hot and dry climate were sensitized to respiratory allergens, in particular house dust
22 89 mite allergens as compared to Gipuzkoa **in the same region nearby** with a more humid climate.
23 90 Peanut allergen Ara h 1 was the most **frequently** recognized class 1 food allergen in
24 91 Northern/Western Europe, while the fruit allergens Pru p 3, Act d 1 and 2 were prominent in
25 92 Southern and Western/Central Europe. Ves v 5-sensitization dominated in North and West/Central
26 93 Europe **at all ages**.
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28 94

29
30 95 **Conclusion:** We show regional, exposome and climate-dependent differences in molecular IgE-
31 96 reactivity profiles in Northern, Western/Central and Southern Europe which may form a molecular
32 97 basis for precision medicine-based approaches for treatment and prevention of allergy.
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35 99
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37 100 **Keywords:** Allergen molecules, IgE-reactivity, Europe, exposome, MeDALL chip, sensitization profile
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103 Introduction

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

110 In 2010, the European Union-funded project "MeDALL" (Mechanisms of the development of
111 allergies) was initiated, a framework for research institutions specialized on various "omics"-
112 technologies to join forces with groups conducting birth cohorts
113 (<https://cordis.europa.eu/project/rcn/96850/factsheet/en>). This gave us the unique opportunity to
114 compare the molecular IgE sensitization profiles from 9 different population-based cohorts located in
115 different geographical regions of Europe; Northern (BAMSE⁵ (Sweden), ECA⁶ (Norway)), West/Central
116 (PIAMA⁷ (the Netherlands), BiB⁸(UK), GINIplus⁹(Germany)), and Southern (INMA¹⁰ Sabadell and
117 Giupuzcoa (Spain) and ROBBIC¹¹ Rome and Bologna (Italy)) Europe. Together these cohorts
118 comprised sera from more than 2800 children between the age of 1 to 16 years, allowing to compare
119 **also to some extent** the evolution of sensitizations from early childhood to adolescence in the
120 different regions of Europe. For this comprehensive IgE-testing, a customized allergen microarray,
121 the MeDALL-chip, was developed that covered 176 allergens and proved superior regarding
122 sensitivity and coverage of allergen molecules as compared to available diagnostic tests.^{12,13} The
123 results of our analysis provide for the first time a comprehensive, high-resolution atlas of IgE-
124 sensitization rates and patterns from the general population from **different regions of** Northern,
125 Western/Central and Southern Europe **followed from early childhood to adolescence**.

127 **Materials and methods**

128

129 **Cohorts and design of the study**

130 IgE measurements were performed retrospectively on sera from 2855 children, aged 1-16 years,
131 from nine different birth cohorts representing the northern, west/central and southern part of
132 Europe. Two cohorts from Northern Europe, BAMSE⁵ (Sweden) and ECA⁶ (Norway), 3 cohorts from
133 Western/Central Europe, PIAMA⁷ (The Netherlands), BiB⁸ (UK), and GINIplus⁹ (Germany), as well as
134 four cohorts from Southern Europe, INMA¹⁰ (Spain, Guipuzcoa and Sabadell) and ROBBIC¹¹ (Italy,
135 Bologna and Rome) were included and information regarding the cohorts can be found in references
136 ⁵⁻¹¹. (Figure 1). For individual cohorts, blood collection had been scheduled for different ages. This
137 allowed us **to some extent** to also investigate IgE sensitization between children of 1, 4, 7-12 and 15-
138 16 years of age. The exact location, participant age and numbers of analyzed sera of each cohort are
139 summarized in Table 1. **Sera were randomly picked within each cohort taking into consideration that**
140 **only sera from children who were born in the region and spent at least the first year of life there**
141 **were analyzed. Furthermore, we aimed at a gender balance regarding the samples. In those cohorts**
142 **where different time points were studied sera were taken from children for whom samples were**
143 **available at each of the time points of sampling. For each of the cohorts ethics approval and written**
144 **informed consent from the parents or legal guardians of the children was available for the analysis of**
145 **allergen-specific IgE⁵⁻¹². The analysis of pseudonymised serum samples was performed at the**
146 **Department of Pathophysiology and Allergy Research, Medical University of Vienna, Austria in a**
147 **centralized manner with permission of the Ethics committee of the Medical University of Vienna,**
148 **EK1641/2014. ~~Randomly picked numbers of sera representative for each cohort were analyzed to~~**
149 **~~avoid biases as much as possible.~~** Possible limitations of the study are mentioned in the discussion
150 section. (<https://www.strobe-statement.org/>).

151

152 **MeDALL-chips**

153 The customized MeDALL-chips were obtained from Phadia Austria GmbH, Part of Thermo Fisher
154 Scientific ImmunoDiagnostics, A-1220, Vienna, Austria. Allergen microarrays were prepared
155 according to the ImmunoCAP ISAC technology with some slight modifications **and had been**
156 **compared with traditional forms of allergy diagnosis in earlier studies^{12, 13}.** More detailed information
157 can be found in the supplementary information about quality controls and subsequent measures
158 (Tables E1-E2).

159

160 **Data analysis**

161 All analyses were performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk,
162 NY, USA). First, allergen molecules were grouped according to their exposure route. We identified a
163 group of respiratory allergens, food allergens and ‘other’ allergens, which induce sensitization via
164 different routes, including insect and latex allergens. Cross-reactive Carbohydrate Determinants
165 (CCD)-bearing allergen molecules were analyzed as a separate group. **Please note that the terms CCD
166 marker and MUXF3 (i.e., Ana c 2.0101) are used in a synonymous manner throughout the manuscript
167 and differ from the term “CCD-bearing allergen” which designate protein allergens containing
168 protein-bound CCDs.** For each cohort and age group, allergic sensitization rates (percentage of IgE-
169 positive subjects) were calculated for each allergen. All allergen molecules were ranked based on the
170 sensitization frequencies and listed by group (Tables E3-E6). The median (minimum-maximum) ISU
171 levels were also provided. From these tables, the 10 highest ranked primary (i.e., non-cross-reactive)
172 allergens were extracted for each cohort and age (Figures 2-5). For these allergen molecules, the
173 percentage of subjects with IgE levels were grouped according to ISU class ranges (low = ≥ 0.3 -1 ISU,
174 moderate = 1-15 ISU, high > 15 ISU).

176 Results

177

178 **Frequencies of detectable molecular IgE sensitization to respiratory allergens and class I** 179 **food allergens vary in the cohorts and increase by age but without major qualitative** 180 **alterations within the cohorts with follow-up samples**

181 Sensitizations to respiratory allergen molecules at four years of age were lowest in the INMA
182 Sabadell cohort and highest in the BiB cohort (Figure 2). Sensitization to house dust allergen
183 molecules at 4 years and 7-12 years were low in the Nordic birth cohorts BAMSE and ECA but
184 frequent in the other birth cohorts (Figure 2). Regarding class I food allergen molecules peanut
185 allergens were frequently recognized in the BAMSE and BiB cohort but not in the other cohorts
186 (Figure 3). Percentages of allergic sensitization and allergen-specific IgE levels increased with age in a
187 similar manner in those cohorts where follow-up samples were available all regions. Serologically
188 detectable IgE sensitization against at least one respiratory allergen increased from 3.7% at 1 year
189 old (in PIAMA) to about 50% in the oldest age group (BAMSE, ECA and GINI) (Figure E1). Food
190 allergens showed a less pronounced increase with age, with a prevalence of 9.3% at age 1 in the
191 PIAMA cohort, which increased to about 15% at 15-16 years of age (BAMSE, ECA and GINI) (Figure
192 E1). Sensitization via other routes (e.g., venom or latex allergy) occurred mostly at an older age.
193 Around 15% of the 15-16 year olds (BAMSE, ECA and GINI) were sensitized to this "other" type of
194 allergen (Figure E1). Despite this quantitative increase, however, no major changes in the
195 qualitative sensitization profiles (i.e., hierarchies of IgE sensitizations) were observed between
196 different age groups (Figures 2-5).

197

198 **Grass pollen allergens are the major pollen allergens in almost all European regions**

199 The top-10 primary respiratory allergen molecules ranked by sensitization rate are shown in Figure 2.
200 Timothy grass allergens were prominent in all cohorts from the age of 4, except in INMA Sabadell. At
201 the age of 7-12 years, children in all cohorts were sensitized to the allergens Phl p 1, 5b, 6, 2, 11 and
202 12 (Table E3). Phl p 1 was the most dominant allergen throughout all the cohorts. However,
203 frequencies of IgE sensitization to Phl p 1 were highest in PIAMA, followed by BAMSE and ECA, and
204 were lowest in the southern cohort ROBBIC. Phl p 7 was recognized in northern and western/central
205 cohorts, but not in southern ones.

206

207 **Sensitization to tree and weed pollen allergens in the different European regions reflects** 208 **the quality of allergen exposure, the exposome**

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2
3 209 The birch pollen allergen Bet v 1 was already an important allergen in the northern cohort BAMSE at
4
5 210 a young age. As much as 12.5% of the 4 year olds had IgE reactivity against Bet v 1. In all other
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7 211 cohorts IgE recognition frequency of Bet v 1 was low. However, frequencies increased with age in all
8
9 212 cohorts for which follow-up samples were available (i.e., ECA, PIAMA, BAMSE). At 12 years of age,
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11 213 Bet v 1 was also recognized by 19% of the children in the west/central cohort PIAMA, and at 15-16
12
13 214 years Bet v 1 was the second or third most recognized marker allergen in all cohorts from Northern,
14
15 215 Central and Western Europe (around 25% in GINI, BAMSE and ECA) (Figure 2).

16 216 In contrast, olive allergen Ole e 1-specific IgE was mainly detected in the southern cohorts.
17
18 217 Both at the age of 4 and 7-12 years, Ole e 1 sensitization was higher in INMA and ROBBIC
19
20 218 respectively, compared to all other cohorts. In addition, the cypress allergen Cup a 1 was prominent
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22 219 in the ROBBIC Rome cohort (Figure 2, Figure 5).

23 220 Regarding weed pollen allergens we found that the major mugwort allergen, Art v 1 was
24
25 221 quite frequently recognized by children from the BAMSE and ECA cohort (Table E3, Figure 2) and the
26
27 222 major Parietaria allergen, Par j 2, showed frequent IgE reactivity in children from the ROBBIC cohort
28
29 223 in Rome which fits to the vegetation profiles in these areas. Interestingly, the major ragweed
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31 224 allergen, Amb a 1, did not seem to be relevant in the cohorts tested by us.

32 225

33 226 **Fel d 1 is an important indoor allergen in almost all European regions whereas frequencies**
34 227 **of sensitization to house dust mite allergens vary considerably**

35 228 The cat allergen Fel d 1 was the most frequently recognized pet allergen molecules in all cohorts and
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37 229 ages except for INMA Guipuzcoa. At 4 years, the sensitization frequency to Fel d 1 was highest in
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39 230 BAMSE (8.7%), BiB (7.2%) and PIAMA (5.6%), whereas it was low in INMA Sabadell (1%) and INMA
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41 231 Guipuzcoa (0.5%) (Figure 2). Around 20% of the oldest children (age 15-16 years) were sensitized to
42
43 232 Fel d 1 in GINI, ECA and BAMSE (Figure 2, Table E3). Sensitization to house dust mite allergens varied
44
45 233 considerably in the different regions. For the western/central and southern cohorts, the house dust
46
47 234 mite allergens Der p 1, 2 and interestingly also Der p 23 were among the allergen molecules with the
48
49 235 highest recognition frequencies (Figure 2). Also, Der p 5, 7, 15, and 37 were often recognized (Table
50
51 236 E3). However, house dust mite allergens were only minor allergens in the northern cohort BAMSE at
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53 237 all ages. However, we also noted striking differences regarding sensitization to house dust mites in
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55 238 Southern Europe. In Sabadell which is close to Guipuzcoa in the same region in Spain, less than half
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57 239 of the children were sensitized to house dust mite allergens (Figure 2). In addition, the fungus
58
59 240 allergen Alt a 1 was prominent only in the southern cohorts. It was the most frequently recognized
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241 respiratory allergen in INMA Sabadell at 4 years and the second most recognized component in
242 ROBBIC Bologna at 7-12 years (Figure 2).

243

244 Genuine sensitization to peanut allergens is frequent only in certain regions

245 The top-10 class 1 food allergen molecules ranked by sensitization rate are shown in Figure 3. The
246 major peanut allergen Ara h 1 was the most frequently recognized **primary class 1 food** allergen in
247 the BiB cohort (6.8%) and the second most recognized in the BAMSE cohort (4.9%) at 4 years. In all
248 other cohorts at this age the sensitization rate was very low. At older ages Ara h 1 was the most
249 recognized allergen molecule in BAMSE, followed by Ara h 2. To a less extent it was also recognized
250 in ECA, but not in western and southern cohorts. Other peanut components like Ara h 3, 6 and 9
251 were recognized in most cohorts, but in lower frequencies (Table E4).

252 In Southern Europe both at the ages of 4 and 7-12 years, as well as in Western/Central
253 Europe, the kiwi allergens Act d 1 and 2 were among the most **frequently** recognized class 1 food
254 allergen molecules, but not in BAMSE. However, the peach allergen Pru p 3 was the dominant class 1
255 food allergen in ROBBIC Rome, but not in ROBBIC Bologna at 7-12 years. Furthermore, the heat-
256 stable and allergenic egg allergen Gal d 1 was most prominent in PIAMA at 1 year. Cow's milk
257 allergens are represented in all but one cohort (INMA Sabadell) at all ages, but mostly in less than 1%
258 of the children (Table E4). Besides class **food 1** allergens, cross-reacting PR-10 proteins like Cor a
259 10401, Mal d 1 and Pru p 1 are among the most frequently recognized molecules in cohorts with high
260 Bet v 1 sensitization rates, due to cross-reactivity (Table E4).

261

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

264 The top-10 of other primary allergen molecules ranked by sensitization rate are shown in Figure 4.
265 Ves v 5 sensitization from a young age was most frequent in the northern cohorts. Ves v 5 was most
266 prevalent in BAMSE at the age of 4 (2.3%). In 7-12 year-old children Ves v 5 sensitization was around
267 7% in BAMSE, ECA and PIAMA, but low in ROBBIC Bologna and ROBBIC Rome. This frequency
268 remained stable at the age of 15-16 years in BAMSE, but increased in ECA (17.5%). In many cohorts
269 the paper wasp allergen Pol d 5 was recognized as well due to cross-reactivity with Ves v 5 (Table E5).

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

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3 277 **Sensitization to ~~carbohydrates~~ is CCD-bearing allergens is dominated by grass pollen nPhl p**
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5 278 **4 in Northern, Central and Western Europe and by ~~cypress~~ nCup a 1 in the south**

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7 279 The timothy allergen nPhl p 4 was the most frequently recognized CCD-bearing allergen in all cohorts
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9 280 and in all age groups, except in ROBBIC Rome and INMA Sabadell (Figure 5). The sensitization rate
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11 281 increased with age in a similar manner in these cohorts (4 years: 2.8 -4%, 7-12 years: 12.9% - 20%,
12
13 282 15-16 years: 19 - 28.9%) (Table E6). Furthermore, the tree-derived CCD-bearing allergen Cup a 1
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15 283 (Cypress) was found to be prominent in the ROBBIC Rome cohort (15.7%) with approximately the
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17 284 double percentage compared to Phl p 4, while sensitization frequencies were low in all other cohorts.
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19 285 ~~The only A frequently~~ recognized CCD-containing food allergen was the walnut allergen Jug r 2. It was
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21 286 ~~recognized detected~~ in all cohorts and age groups, mostly in relatively low frequencies ($\leq 4.5\%$).
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23 287 ~~Interestingly, sensitization rates to the pure CCD marker MUXF3 were similar in all cohorts and rather~~
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25 288 ~~low (i.e., approximately 1-2%) (Table E6).~~
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290 Discussion

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

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

300 ~~Grass pollen allergen sensitization dominated in most of the European regions, while IgE reactivity to~~
301 ~~tree pollen was in a higher degree region dependent. The major cat allergen, Fel d 1, was an~~
302 ~~important indoor allergen in almost all European regions in contrast to the house dust mite allergens~~
303 ~~Der p 1, 2 and 23 where the sensitization frequencies varied considerably between regions.~~
304 ~~Differences were also observed for class 1 food allergens. The major peanut allergen Ara h 1 was the~~
305 ~~most recognized primary allergen in the BiB cohort and second most recognized in the BAMSE cohort~~
306 ~~at 4 years, while in Southern and in Western/Central Europe, fruit allergens such as Pru p 3, the kiwi~~
307 ~~allergens Act d 1 and 2, but also egg allergens were among the most recognized allergen molecules.~~
308 ~~By contrast, sensitization to class 2 food allergens causing mainly oral allergy syndrome such as the~~
309 ~~Bet v 1-related PR10 allergens was tightly linked to primary sensitization to the corresponding~~
310 ~~respiratory allergens. Interestingly, wasp allergen Ves v 5 and other insect allergens are dominant~~
311 ~~allergen molecules in Northern and Western/Central Europe at all ages, but not in Southern Europe.~~

312 ~~While the timing of sensitization onset was very similar in the different European regions,~~
313 ~~many~~ Striking regional differences regarding molecular IgE sensitization profiles in the different
314 cohorts were observed ~~could be observed in the recognition of allergens~~. There are only few
315 previous studies which have analyzed molecular IgE sensitization profiles in population-based cohorts
316 from individual countries (e.g., UK, Germany, Italy) which in fact confirm the molecular sensitization
317 patterns which we observed for these countries^{14, 15, 16}. However, there is only one study which
318 involved different regions of France and demonstrated that there can be important differences
319 regarding molecular sensitization profiles ~~have in an unambiguous manner shown that these~~
320 ~~differences can be explained by the~~ based on differences in the regional exposome. ~~E.g.~~In fact,
321 Siroux et al showed that the sensitization profile of people from five different regions even within on
322 country (i.e., France) differed significantly, which was reflected in the differences in vegetation

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3 323 between the studied areas.¹⁷ Also in our study the exposome and in particular the climate seemed to
4 324 show local differences as observed between the cities of Rome and Bologna **as well as Sabadell and**
5 325 **Gipuzkoa** in Italy and in Spain, **respectively**.

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8 326 The fact that the climate in Sabadell is much more hot and dry¹⁸ than in Gipuzkoa ~~which are~~
9 327 ~~both located in the same region not far from each other in Spain~~¹⁹ may be a reason why less than half
10 328 of the children of the same age (i.e., 4 years) were sensitized to respiratory allergens, **in particular to**
11 329 **house dust mite allergens**. Thus frequencies of IgE sensitization to respiratory allergens in children at
12 330 four years were especially low in the dry **and hot** region of Sabadell as compared to cohorts from
13 331 North-, West- and Middle Europe.

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18 332 When scrutinizing differences between the cohorts, we first observed that Phl p 1, the major
19 333 timothy grass pollen allergen, was the ~~most~~ dominant allergen in all investigated regions due to the
20 334 ubiquitous distribution of grasses. Phl p 1 has been suggested to initiate the sensitization process to
21 335 timothy grass in pollen allergic children.^{20, 21} **Furthermore**, Phl p 1 is highly cross-reactive with group 1
22 336 allergens in different grass species and unlike other grass pollen allergen groups, group 1 allergens
23 337 occur in all grass species²², which is reflected in the high frequency of sensitization against grasses in
24 338 general in all regions. However, sensitization against Phl p 1 and other timothy grass allergens were
25 339 not detected in subjects of the INMA Sabadell cohort. Again, this is likely due to the dry, ~~but~~ **and hot**
26 340 **and Mediterranean** climate there.²³

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32 341 For tree **pollen** allergens significant differences in sensitization profiles were found between
33 342 Northern/Central and Southern Europe, which clearly reflect the different tree exposomes in these
34 343 regions. Birch trees are most common in Northern and Central Europe.^{24, 25} In line with this, Bet v 1,
35 344 the major birch allergen, was **already** prominent in the BAMSE and ECA cohorts (Northern Europe)
36 345 ~~from~~ **at** a young age, while it played a more significant role in PIAMA and GINI (Western/Central
37 346 Europe) in older children **suggesting an increase of detectable IgE sensitization by age**. However, **for**
38 347 **most of the cohorts we did not have follow up samples to draw firm conclusions regarding the**
39 348 **longitudinal development of IgE sensitizations and the associated development of symptoms**. Such
40 349 **studies have been performed so far only for certain allergen sources and in certain cohorts**²⁶⁻³⁰ **and**
41 350 **were not the topic of our study which aimed to provide a comprehensive picture of molecular IgE**
42 351 **sensitizations in different regions of Europe**.

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47
48 352 In contrast to Northern Europe, Italy and Spain, where olive trees are responsible for a significantly
49 353 part of airborne pollens³¹, sensitization was observed in the INMA and ROBBIC cohorts. Cypress is
50 354 another typical Mediterranean tree found above all in Italy³² which was reflected in the dominance
51 355 of Cup a 1 sensitization mainly in Rome. **Like for Gipuzkoa and Sabadell, two close regions in Spain we**
52 356 **noted strong differences regarding molecular IgE sensitization profiles between Bologna and Rome**.

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3 357 In Bologna sensitizations to grass pollen allergens dominated whereas in Rome sensitization to HDM
4 358 allergens were more frequent. We think that it is an important finding of our study that we detected
5 359 strongly varying molecular IgE sensitization profiles even in regions which are close to each other
6 360 within one country because this finding has important implications for precision medicine
7 361 approaches such as allergen avoidance (e.g., HDM allergy) and accurate prescription of allergen-
8 362 specific immunotherapy. Molecular diagnosis is especially important for the precise identification of
9 363 the genuinely sensitizing allergen sources which can be obscured by cross-reactivity when allergen
10 364 extracts are used.

11 365 The major cat allergen Fel d 1 was the most prominent frequently recognized allergen among
12 366 the furry animals. When comparing the European regions, Fel d 1 sensitization was most common in
13 367 Northern and Central Europe already from a young age, while sensitization frequencies were lower in
14 368 Southern Europe. A similar profile was recently also described for the Moscow region of Russia,
15 369 where Fel d 1 was the most frequently recognized indoor allergen.³³ The data is in line with a report
16 370 showing that a higher percentage of people in Norway, Sweden and the UK had a cat during
17 371 childhood.³⁴ However, since multiple factors have been found to affect Fel d 1 levels, including
18 372 keeping cats indoors, smoking habits and ventilation, it still remains unclear why Fel d 1 levels in
19 373 house dust are lower in southern Europe.³⁵ One possibility though may be that cats are less often
20 374 kept indoor in these countries due to the climate.

21 375 The presence of house dust mite allergens, both Der p and Der f, depends on humidity.^{36, 37}
22 376 This is in line with our finding that IgE to the house dust mite allergens were almost absent in BAMSE,
23 377 and very low in Sabadell, present in ECA and PIAMA, but and most prominent in the BiB cohort from
24 378 the UK. In most cohorts sensitization was observed against several of the major house dust mite
25 379 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
26 380 10.³⁸ Unlike house dust mites, the fungus *Alternaria alternata*, has shown to be an indoor allergen
27 381 which grows better in a dry and warm climate.²² As a result, Alt a 1 was found to be one of the most
28 382 important allergens only in the cohorts from Sabadell (Spain) and Bologna (Italy).

29 383 Regarding food allergens our study differs from the EuroPrevall study which has focused on
30 384 food-allergic subjects and only few molecular analyses focusing on certain food allergens have been
31 385 performed within EuroPrevall³⁹. By contrast, our study has investigated random population samples
32 386 from different parts of Europe for IgE sensitizations to food allergens. We found that Ara h 1 and 2
33 387 are clearly the most prominent allergens in BAMSE and BiB, but rare in the other cohorts.
34 388 Geographical differences in clinical and immunological profiles of peanut allergens have been
35 389 reported. Vereda et al. showed that peanut allergic patients from the US and Sweden recognized the
36 390 storage proteins Ara h 1-3 more frequently compared to Spanish patients who were more often

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3 391 sensitized against the lipid transfer protein Ara h 9.⁴⁰ We also noted that Ara h 9 sensitization was
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5 392 higher in the southern cohorts INMA Gipuzkoa and ROBBIC Rome compared to BAMSE. These
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7 393 differences are not only depending on the amount and timing of peanut consumption. A study from
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9 394 Sweden has shown that the increase in peanut sensitization over the years is not only due to
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11 395 increased peanut consumption.⁴¹ Differences in preparation of peanuts also plays a role. Roasted
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13 396 peanuts, which are consumed more in Sweden, the US and other western countries, contain more
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15 397 stable proteins and thus may have a higher allergenicity.⁴² Regarding peanut differences of allergen
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17 398 contact via the skin may also be considered to be responsible for different sensitization rates in the
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19 399 different populations –Sensitization against other food allergens depended mainly on besides
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21 400 nutritional habits.⁴³ High sensitization rates to peanut allergens and to the major fish allergen Gad c 1
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23 401 in the BiB cohort from UK as compared to other cohorts may be an example for such nutritional
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25 402 habits. However, sensitization against the dominant shrimp allergen Pen m 1 may reflect to some
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27 403 extent cross-reactivity with the tropomyosin Der p 10. In individual patients, specific IgE levels to Pen
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29 404 m 1 and Der p 10 and IgE cross-inhibition studies may inform which allergen may have been the
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31 405 genuinely sensitizing molecule. Act d 1 sensitization was found to be prominent only in southern
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33 406 Europe, where kiwifruit is grown locally, and especially Italy is known for its high kiwifruit
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35 407 consumption.⁴⁴

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37 408 Regarding venom allergens, our study provides new and unexpected information, since data
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39 409 on hymenoptera IgE sensitization are scarce, especially in children. We found that between 7 and
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41 410 20% of 15-16 year olds from the northern cohorts showed IgE-reactivity against the major wasp
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43 411 venom Ves v 5 while a considerably lower rate of sensitization was found at younger ages, which is in
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45 412 line with data reported previously.⁴⁵ Although we did not have data from Southern Europe for the
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47 413 15-16 year olds, Ves v 5 sensitization seems to be less frequent in this area at a younger age. The
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49 414 most important wasp species, belonging to the *Vespula* genus and responsible for Ves v 5
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51 415 sensitization, have been found to be present all over Europe, but more precise data on their
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53 416 geographical distribution and population density are lacking, which makes it difficult to explain the
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55 417 observed differences in sensitization frequency.⁴⁶ We speculate that children in Northern Europe
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57 418 could be more exposed to wasps, for example because they spend more time outdoors and in nature
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59 419 during the summer period.

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61 420 With respect to IgE-positivity to natural allergen molecules bearing cross-reactive
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63 421 carbohydrate determinants (CCDs), similar rates were observed throughout all regions of Europe,
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65 422 with Phl p 4 being the most prominent CCD-bearing allergen. For these CCD-bearing allergen
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67 423 molecules, coming mainly from plants, it is difficult impossible to distinguish IgE-reactivity to the
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69 424 sugar moieties from antibody-binding to the protein backbone at an individual level. However, only

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3 425 in southern cohorts IgE-levels to nCup a 1 were found to be indicative for true sensitization to those
4 426 trees (cypress, cedar) or grasses (Bermuda grass) that are native in those regions. The remarkably
5 427 high prevalence of IgE-positivity to nJug r 2 in the German GINI-cohort can presumably be partly
6 428 attributed to reactivity with CCDs present on this glycoprotein, while in other cohorts reactivity to
7 429 nJug r 2 was paralleled by an increase of IgE to Jug r 1, indicative of genuine IgE-sensitization to
8 430 walnut. Regarding the only CCD marker (i.e., MUXF3) which was tested in each of the cohorts a
9 431 relatively low frequency (approximately 1-2%) of IgE reactivity was found indicating that for CCD-
10 432 bearing allergens also protein IgE epitopes play a role.

11 433 It is one limitation of our study that not all children from whom sera had been collected had
12 434 exactly the same age from the same time points had not been collected in each of in the investigated
13 435 cohorts but this should not affect the major findings of the study which are that sensitization profiles
14 436 to allergen molecules seemed to vary regarding the allergen exposome and climate in the different
15 437 cohorts and remained largely unaltered over time. Another limitation of our study is that we have
16 438 not taken into account the atopic background of the parents of children when picking the serum
17 439 samples from children but it seems that the atopic background of parents does not have such strong
18 440 effects on allergic sensitization in children⁴⁷. Likewise, we have not stratified children according to
19 441 genetic background, ethnicity, nutritional habits and environmental pollution. However, in a recent
20 442 study we did not find much evidence that pollution would influence allergen-specific IgE
21 443 sensitization⁴⁸. Other limitations of our study are that we make only descriptive comparisons without
22 444 any adjustments and that the analyses were done only for available samples for arbitrarily selected
23 445 cohorts. On the other hand one may consider the arbitrary analysis of children who were born and
24 446 grew up in a region as a strength because it may provide real-life pictures of the local molecular
25 447 sensitization profiles. NeverthelessFurthermore, to the best of our knowledge, our study revealing
26 448 molecular sensitization profiles in a population-based cohorts of children from a continent
27 449 represents the first of its kind in the world. A more detailed molecular IgE sensitization map of
28 450 Europe and other continents may be obtained in the future by cross-sectional analyses of random
29 451 populations of patients who are recruited by questionnaires from several different regions of the
30 452 individual countries with different climate and living habits. Like in our study the patients should
31 453 have been born and grown up in the regions of investigation to inform about the influence of the
32 454 exposome and climate conditions on allergic sensitization.

33 455 In conclusion, this comprehensive data-set of high-resolution IgE-sensitization patterns of
34 456 several thousand children from population-based European birth cohorts, with a north, south and
35 457 west/central gradient, provides a detailed overview of regional and age-dependent differences in
36 458 IgE-reactivity profiles of the general populations, which depend largely on the local exposome and

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3 459 climate. ~~Although there were different age groups no major changes in the sensitization profiles~~
4 ~~were noted.~~ Since the method used for IgE-detection was based on a commercially available
5 460 platform (ImmunoCAP ISAC), our data can be combined with existing and future data-sets from
6 461 further cohorts based on this technology. Furthermore, our sensitization map of Europe may form a
7 462 basis for molecular strategies for prevention and therapy of allergy.
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13 465 **Authors contribution:**

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15 466 CL, JA, JB and RV designed the study. CL and RK performed the experiments. CL and GK analyzed and
16 467 interpreted the data. IK, EM, MW, K-HC, K L-C, DP, DG, HAS, RB, UG, MS, JH, DW, JW, SV, SW, AB
17 468 collected patients' material and/or prepared and characterized allergen molecules. CL, GK, MvH, RV
18 469 contributed to data interpretation and wrote the first draft of the manuscript. All authors critically
19 470 reviewed the manuscript and approved the submitted version.
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25 472 **Conflicts of interest:** R.V. receives research grants from HVD Biotech, Vienna, Austria and Worg
26 473 Pharmaceuticals, Hangzhou, China. He serves as consultant for Worg and Viravaxx AG, Vienna,
27 474 Austria. MvH has received lecture fee from Thermo Fisher Scientific. GK has no conflict of interest to
28 475 declare. CL and SW are currently employees of MacroArray Diagnostics GmbH, Vienna, Austria. JB
29 476 reports personal fees from Cipla, Menarini, Mylan, Novartis, Purina, Sanofi-Aventis, Teva, Uriach. He
30 477 is shareholder of KYomed Innov and MASK-air-SAS. The rest of the authors report no conflict of
31 478 interest.
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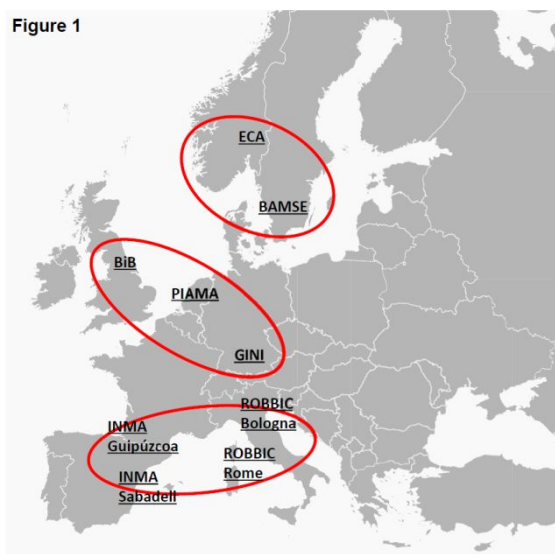
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644 **Figures and legends**

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

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Table I

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

649
650 Table I. MeDALL-cohorts and numbers of samples analyzed with the MeDALL-chip. Samples obtained
651 at 7-12 years (purple boxes) or at 15-16 years (yellow boxes) were combined in age groups.

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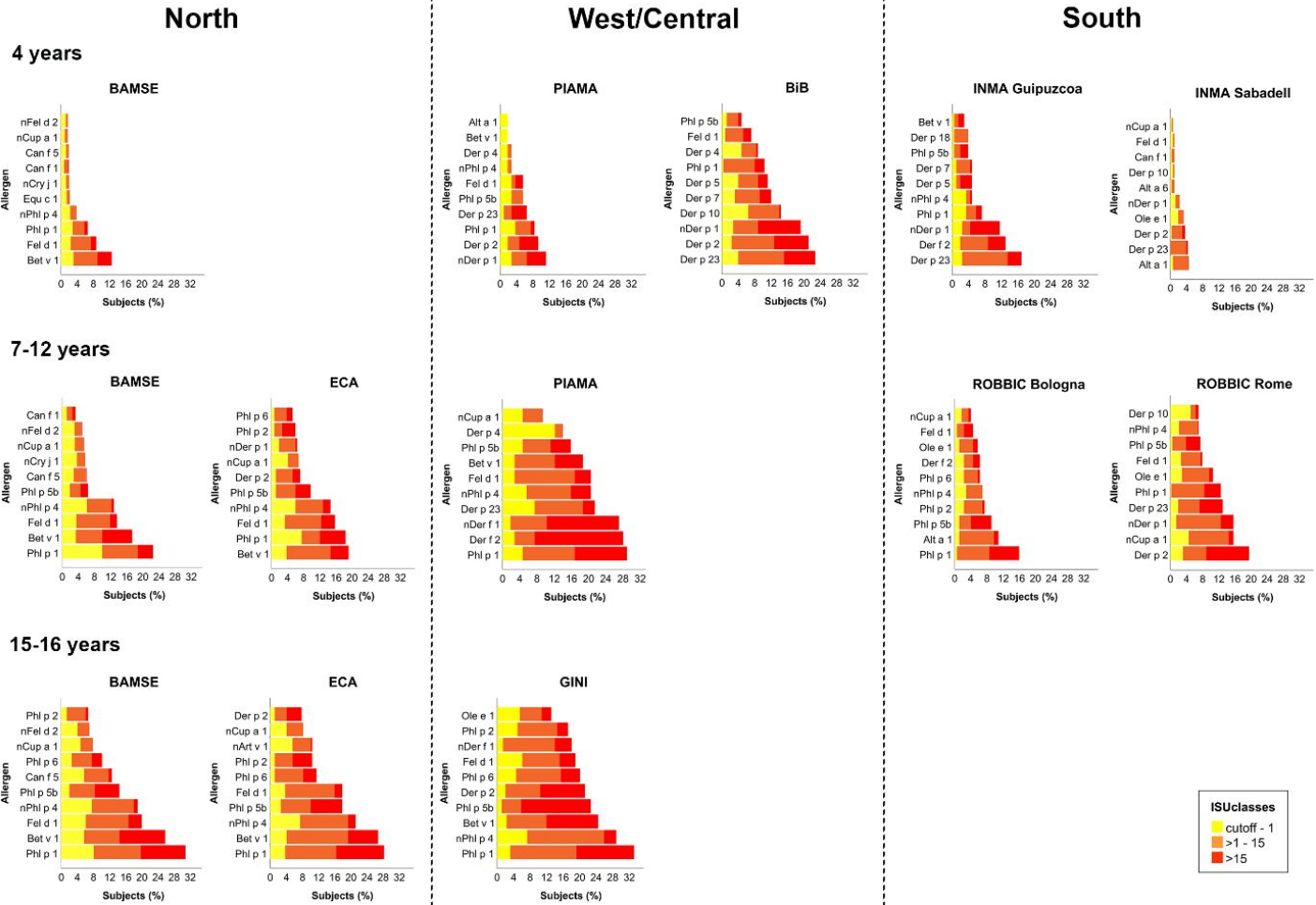
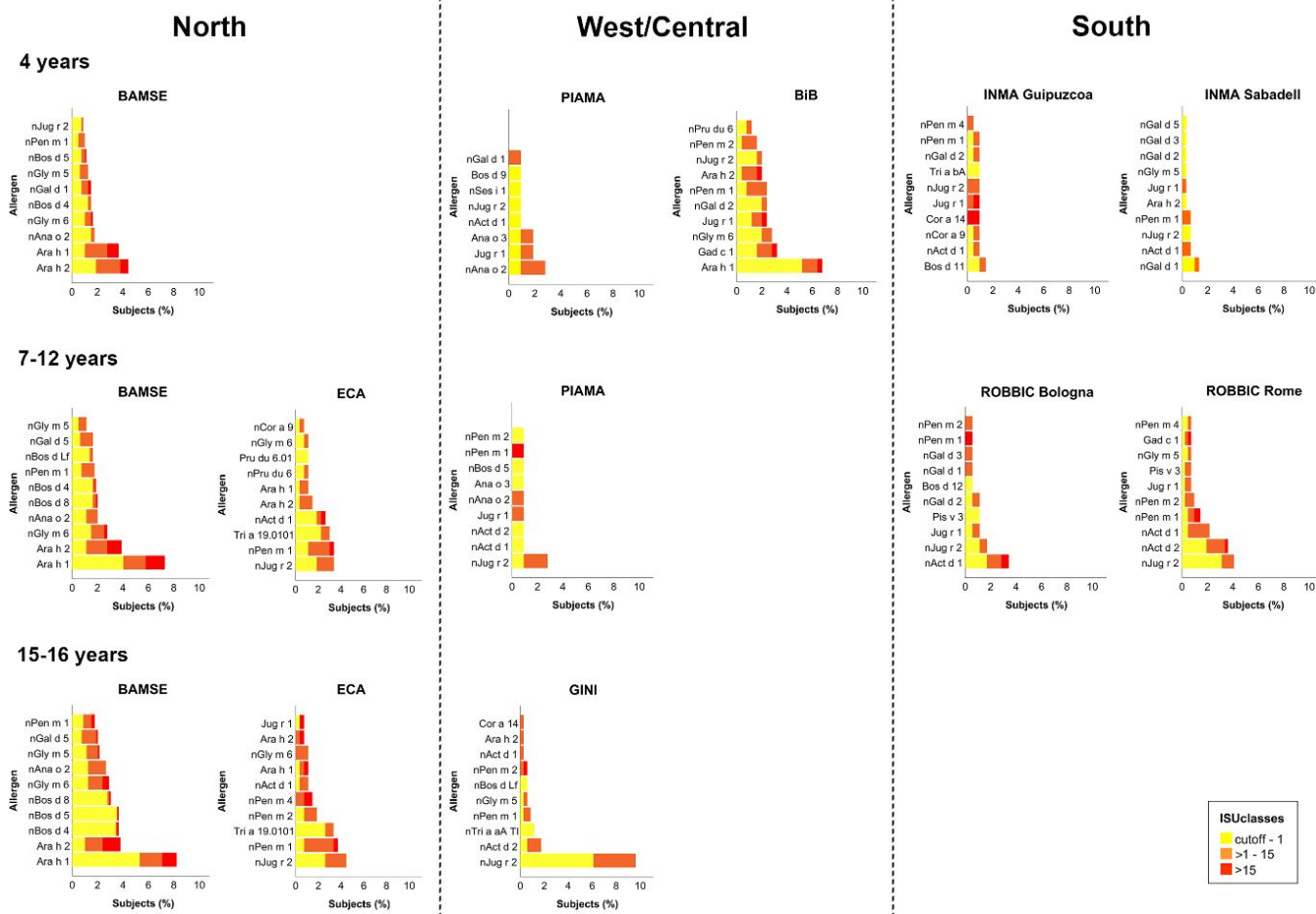


Figure 2. Overview of the 10 most frequently recognized primary respiratory allergens per cohort. The cohorts are organized based on age and region. For each cohort, allergens are ranked based on sensitization rate. Each bar shows the percentage of subjects with IgE levels within the different ISU classes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

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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).

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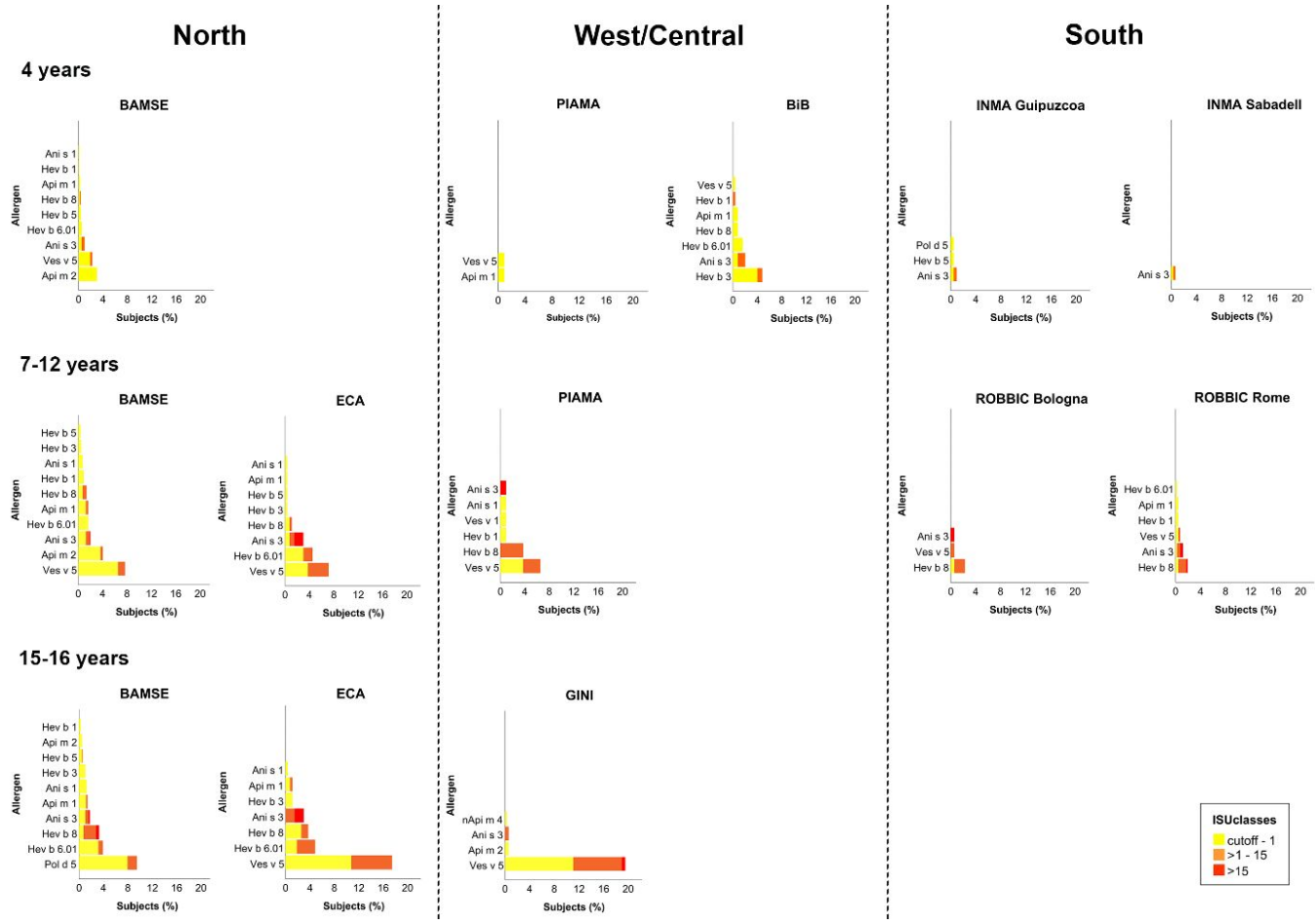


Figure 4. Overview of the 10 most frequently recognized primary other allergens per cohort. The cohorts are organized based on age and region. For each cohort, allergens are ranked based on sensitization rate. Each bar shows the percentage of subjects with IgE levels within the ISU classes (yellow =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

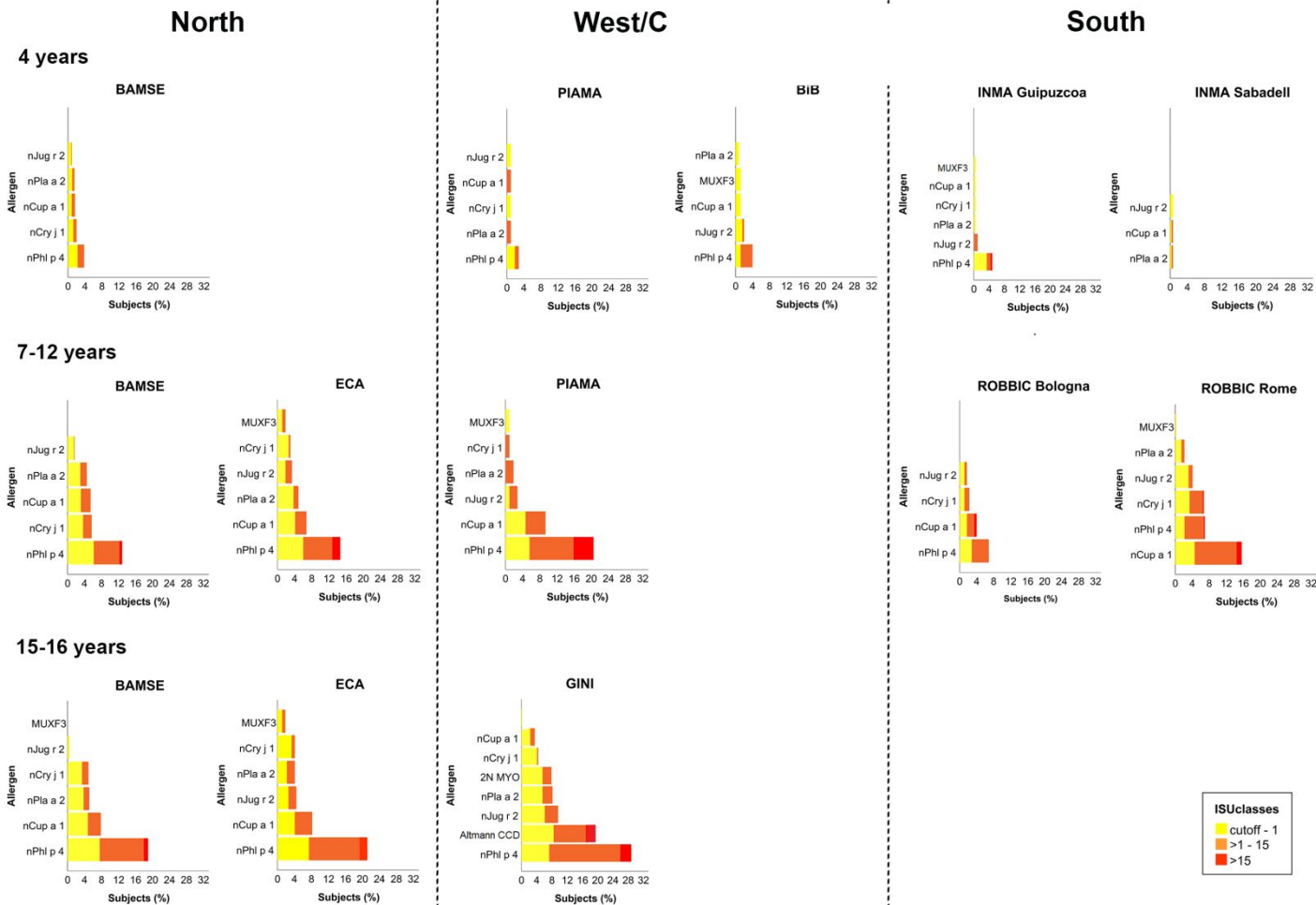


Figure 5. Overview of the most frequently recognized CCD-bearing allergens per cohort. The cohorts are organized based on age and region. For each cohort, allergens are ranked based on sensitization rate. Each bar shows the percentage of subjects with IgE levels within the ISU classes (yellow = ≥ 0.3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