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**Cross-sectional study on allergic sensitization of Austrian adolescents
using molecule-based IgE profiling**

Short title: IgE sensitization study of Austrian adolescents

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ABSTRACT

Background

Allergen specific IgE antibodies are a hallmark of type I allergy. The aim of this cross-sectional study was to analyze the sensitization profiles of an Austrian adolescent population utilizing molecule-based IgE diagnosis.

Methods

Serum samples of 501 non-selected pupils from Salzburg, Austria, were tested in ImmunoCAP ISAC® for IgE reactivity to 112 single allergens. Sensitization profiles were assessed and statistically coordinated with reported allergies.

Results

In the population aged 12-21 years, 53.5% showed IgE reactivity to at least one allergen tested. The highest prevalence was found for Phl p 1 from grass pollen (26.5%), group 2 mite allergens (18.2%), Bet v 1 from birch pollen (16.3%) and Fel d 1 from cat (14.4%). The majority of participants showed a complex sensitization profile and reacted on average to 9 allergens. Pollen sensitization was highly prevalent (41.7%) and mainly driven by group I grass and PR-10 allergens of the birch family, while Pla l 1 represented the most relevant weed. Diagnosed and self-reported allergies were noted in 21.8% and 55.5% of participants, respectively and correlated well with *in vitro* results. Among atopic individuals, 71.4% reported to suffer from at least one allergy; concordance was found for grass and cat sensitization while venom and weed pollen positive individuals were frequently asymptomatic.

Conclusions

More than half of the tested adolescent population had already established an atopic status presenting a complex IgE reactivity profile dominated by pollen sensitization. Detailed molecule-based analysis allows determining relevant biomarkers and monitoring of the atopic status in populations.

INTRODUCTION

The presence of allergen specific IgE antibodies is one of the prerequisites for the development of type I allergies (1). Recently, significant increases in the prevalence of allergic symptoms, affecting up to 20% of the global population in 2013, have been observed in several studies (2-5). The investigation

of IgE sensitization in population-based epidemiologic studies is therefore of high relevance (6). It enables a more efficient selection of tests for routine allergy diagnostics with respect to varying patterns in different geographical regions and distinct allergen exposure (6). In Austria, located in central Europe, a population-based sensitization prevalence of 50.8% was reported in Viennese adults back in 1995 performing skin prick tests with extracts of inhalant allergens (7). So far, epidemiologic studies on IgE sensitization at the molecular level have not been performed in our geographic region.

Sensitization, hence specific IgE levels have been positively associated with the risk of allergen-related symptoms (8). Molecule-based diagnosis is the method of choice for the detection of allergen-specific IgE antibodies at the allergen component level (9). Assays using purified allergens allow a more detailed examination of IgE cross-reactivity, risk molecules and prognostic sensitizations (4). Molecule-based diagnosis avoids problems associated with allergen extract tests, such as heterogeneous allergen content, cross-reactivity with allergens of low clinical relevance or the widespread presence of carbohydrate determinants (10). It showed a better predictive value for allergy symptoms compared to the use of extracts, and also directly impacted the prescription of allergen-specific immunotherapy (11, 12). For the simultaneous determination of IgE antibody levels against a large number of natural and recombinant purified allergens, microarray technologies offer a valuable approach (13). The ImmunoCAP ISAC 112[®] enables the detection of specific IgE to 112 allergens from 51 sources in a small volume of one single serum sample. This multiplex assay was shown to be a repeatable and reproducible *in vitro* diagnostic tool for the determination of allergen-specific IgE (14). Using multiplex assays for IgE detection in epidemiological studies allows for a comprehensive evaluation of sensitization profiles without preselection (15). Investigating a specific cohort of a defined age group enables to focus on manifestations at a certain age window. In the adolescent age, allergic reactions to food allergens from early childhood usually disappear in the course of the allergic march, while sensitizations to environmental allergens might already be manifested (16).

The aim of the presented population-based study was to analyze the IgE sensitization profiles of adolescents in the region of Salzburg (Austria) using the ImmunoCAP ISAC[®]. Investigating samples from young people provided an insight on the first manifestation of persistent allergic sensitizations and enables to gather information on sensitization patterns. Using a multiplex array with purified recombinant and natural allergen molecules permitted the establishment of detailed sensitization profiles, which were linked to information on reported allergies.

METHODS

Study design and participants

This cross-sectional study aimed to investigate IgE sensitization profiles of ≥ 500 non-selected students from schools in the region of Salzburg, Austria. Students were enrolled for a one-time sampling between October 2013 and May 2014. Participants were recruited from the school grades 8 to 13 (expected age range 13-19 years) in information meetings and no exclusion criteria were applied. Written informed consent from the participating pupils and their legal guardian (if they were underage) was obtained. The study was conducted according to common ethical principles and approved by the local ethics committee of Salzburg, Austria, Nr. 415-E/1669/6-2013. All participants filled out a specifically developed written questionnaire which was anonymous and linked to the IgE data using a number code. They were asked to indicate doctors' diagnosed as well as self-reported allergies, and further specify if they react to grass pollen, tree pollen, weed pollen, cat hair, dog hair, house dust mite, food or "other sources". Study design and interpretation is following recommendations of the STROBE Initiative (17).

Blood sampling and specific IgE analysis using an allergen multiplex array

Capillary blood samples were obtained from the fingertip, incubated at room temperature for 15 min and centrifuged at 14,000 rpm. Serum was separated from the blood cells and the serum samples were stored at 4°C for transport and at -20°C until further analysis. Sera were analyzed for specific IgE to 112 single purified allergens using the ImmunoCAP ISAC[®] (Thermo Fisher Scientific, Uppsala, Sweden). The test was performed according to the manufacturer's protocol with 30 μ l of serum

(Protocol No. 20-01-02-6). Resulting fluorescent signals were measured using a confocal laser scanner (LuxScan-10K, CapitalBio, Beijing, China) and data were analyzed in Phadia Microarray Image Analyzer software to be transformed into semi-quantitative ISAC Standardized Units (ISU). Specific IgE values of ≥ 0.3 ISU were considered positive.

Statistical analysis

Assuming a 25-35% prevalence for allergic diseases in the general population, a sample size of $n \geq 500$ allows for a confidence interval error margin of 4 percentage points when estimating overall prevalence. Error margins are larger for subgroups determined by demographic and diagnostic factors or combinations of those. Statistical analysis of data was performed in Prism 5 for Windows (GraphPad Software, Inc., La Jolla, CA, USA) and R (18). Data for Venn diagrams were transferred to Microsoft Excel (Microsoft Corporation) and shown with Adobe Illustrator CS6 (Adobe Systems Incorporated). Correlations between specific ISU values were calculated as Spearman's rank correlation of sensitized individuals, where ρ 0.5-0.7, 0.7-0.9, and 0.9-1.0 is interpreted as moderate, high, and very high correlation, respectively (19). ISU distributions between different groups were compared using the two-sample rank sum (or Wilcoxon-Mann-Whitney) test, contingency data was analyzed by Fisher's exact test for count data. P-values ≤ 0.05 were considered statistically significant. To analyze sensitization profiles, set diagrams (Venn diagrams) were generated according to data from the assessment of specific IgE titers. Groups containing ≥ 3 subjects are depicted in set diagrams and details on groupings of allergens are provided in Supplementary Figure 1. To assess associations between ISU to single allergens and allergies, the nonparametric relative effect was estimated per allergen. Estimated probabilities > 0.6 were considered as strong association. Inference was performed using a Bonferroni-Holm multiple testing correction.

RESULTS

Sensitization prevalence

Serum samples and questionnaires were obtained from 501 of 659 randomly contacted students, translating to an overall participation rate of 76.0% (Fig 1a). Additional data on the study population

are depicted in Fig 1b. Among 501 participants, 268 demonstrated IgE reactivity to at least one allergen which corresponds to an atopy frequency of 53.5%. Testing 112 components on the allergen microarray resulted in positive IgE recognition of 76 allergens from 16 different allergen sources as well as the cross-reactive carbohydrate determinant (CCD) marker MUXF3 (Fig 2). Reactivity of single atopic individuals was directed against 1 to 33 different components. On average, individuals reacted to 8.6 single allergens from 3.6 different sources. No age dependency was found for IgE sensitization frequency or number of positively tested allergens. Highest sensitization prevalence was found to allergens from grass, tree and weed pollen as well as from mites and cats (Fig 2). Highest prevalence regarding single allergens was found for grass allergens Phl p 4, Cyn d 1 and Phl p 1. Highest median IgE levels were detected for Phl p 5, Der p/f 2, Phl p 1, Bet v 1, Phl p 2/6, and Pla l 1. Relevant levels were further detected for Amb a 1, Alt a 1 and Phl p 7, although these allergens were low in sensitization prevalence (Suppl. Fig 2).

IgE sensitization profiles

To obtain a comprehensive sensitization profile, overlapping IgE reactivity was analyzed and depicted in set diagrams. The majority of atopic individuals showed a complex multi-sensitization profile and sensitization to food, latex and animals typically coincided with pollen allergens (Fig 3a). Exclusive IgE reactivity to pollen, mites and venom was observed in 48, 22 and 22 participants, respectively. Interestingly, individuals exclusively reacting to pollen allergens showed significantly lower IgE levels to pollen allergens, *i. e.* PR-10, grass group 1, profilins and Ole e 1-like proteins ($p < 0.01$) compared to subjects reacting to pollen and ≥ 3 other sources. Analogous, individuals reacting exclusively to insect venom and mites demonstrated significantly lower IgE levels for Api m 1 and Der p 10, respectively, compared to multi-reactors.

Among pollen-sensitized subjects ($n=209$) (Fig 3b), grass pollen allergens were dominant either individually ($n=47$) but more often in context with other pollen allergens ($n=130$). Isolated reactivity to Betulaceae, olive, plantain or mugwort pollen was observed at low frequencies. Sensitization to goosefoot, plane tree, cypress and cedar was generally low and coincided with grass pollen

sensitization. Among grass pollen-sensitized individuals (n=178) (Fig 3c), highest prevalence was observed for Phl p 4 (n=139), Cyn d 1 (n=137) and Phl p 1 (n=133). These allergens showed a vast overlap in sensitization; IgE reactivity to other grass pollen allergens was only found within this group. Interestingly, 23 individuals showed IgE reactivity to Cyn d 1 in the absence of Phl p 1 reactivity, whereas Phl p 5 was highly associated with Phl p 1.

Sensitization to tree pollen allergens was mainly driven by Bet v 1 (82/142 subjects) and homologous pathogenesis-related (PR)-10 proteins from alder and hazel pollen (Fig 3d). Ole e 1-positive individuals were mostly also sensitized to PR-10 proteins, while 15 individuals reacted exclusively to Ole e 1. Sensitization patterns to weed pollen allergens (n=103) (Fig 3e) were generally diverse and sensitization to Pla l 1 (n=52) was the most dominant followed by Art v 1 (n=36). Interestingly, only partial overlap with other Ole e 1-like homologs was found for Pla l 1 as well as Ole e 1 (Fig 3f).

IgE reactivity to food allergens was mainly driven by PR-10 proteins (72 of 113 food allergen sensitizations) which are categorized as class 2 food allergens (Fig 4a). Analyzing class 1 food allergens, which possess the capacity for primary sensitization, Jug r 2 from walnut, Pen m 2 from shrimp and Act d 2 from kiwi were most frequently detected. The relevance of allergic sensitization to hen's egg, milk or wheat was negligible in our cohort (Fig 2).

Sensitizations to mite allergens were mainly elicited by group 2 and group 1 mite allergens (Fig 4b). A complete overlap was found for Der p 2 and Der f 2, while Der f 1 was tested positive with slightly higher frequency compared to Der p 1. Sixteen and 29 participants demonstrated exclusive reactivity to group 1 and group 2 mite allergens, respectively. Sensitizations to other mite allergens (Lep d 2, Blo t 5 and Der p 10) mainly appeared in combination with the major allergens but rarely in combination with each other. Among tested insect venoms, the most frequent reactivity was found for Ves v 5 scoring positive in 10.6% of individuals (Fig 4c). Overlapping sensitization between group 5 allergen from wasp and group 1 allergen from bee was very low; 33 and 22 subjects were mono-reactive to Ves v 5 and Api m 1, respectively. The vast majority of IgE reactivity in the group of animals was

attributed to Fel d 1 (n=72), sensitization to other animal allergens was generally rare and mostly associated with Fel d 1 reactivity (data not shown).

Correlation patterns of IgE reactivities

Spearman correlations between ISU values to single allergens were analyzed to identify coherences between sensitizations. A very high correlation was found within group 1 and group 2 mite allergens of *D. pteronyssinus* and *farinae* ($\rho > 0.933$), and high correlation between group 1 and 2 mite allergens ($\rho > 0.602$). As anticipated, very high to moderate correlations ($\rho > 0.5$) were also found for allergens of the same protein family, e. g. profilins, PR-10, grass group I allergens, polcalcins, and lipocalins. Similar values ($\rho > 0.527$) were interestingly also observed for non-related allergens of grass pollen. Sensitization frequency as well as ISU levels within the PR-10 family (Fig 5) were highest with Bet v 1, followed by Cor a 1.0401, Aln g 1, Mal d 1, Cor a 1.0101, Pru p 1 and Ara h 8. Lower levels were obtained with Gly m 4, Api g 1 and Act d 8. These individuals were all IgE-reactive to pollen PR-10 except one who was exclusively sensitized to Pru p 1.

In our cohort, 22 participants showed IgE reactivity to the CCD marker MUXF3. Glycan-specific reactivity correlated with allergens from natural as well as recombinant sources ($p < 0.05$, $\rho > 0.2$), and mainly involved allergens from the profilin, Ole e 1-like, pectate lyase and grass group 1 protein family. Highest CCD correlation was found for Jug r 2 ($p < 0.001$, $\rho = 0.584$) and Pla a 2 ($p < 0.001$, $\rho = 0.481$). Interestingly, we found an inverse relation for Ves v 5, which negatively correlated with allergens from grass pollen (Phl p 1/5) and mites (Der p/f 2). A negative correlation was also found between Der p 1 and Phl p 6 ($p < 0.05$, $\rho = -0.142$).

IgE sensitization and allergies

In order to relate IgE sensitization with allergic symptoms, subjects reported ~~clinically~~ doctors' diagnosed and self-reported allergies ~~symptoms~~ in a questionnaire. Answers from 466 participants indicated that 21.9% suffered from a diagnosed allergy to at least one allergen source. Allergic reactions to grass pollen (n=65), mites (n=35), tree pollen (n=33) and cat hair (n=27) were most

frequently listed. Diagnosed allergies clearly correlated with an atopic status ($p < 0.0001$) compared to those without allergies. In general, more than 92.2% of subjects with diagnosed allergies also showed IgE sensitization with molecule-based diagnostics. From all 112 allergens present on the chip, Phl p 1 was identified as strongest discrimination factor for development of allergies.

Self-reported allergies to specific allergen sources as well as unknown elicitors were stated by 55.5% of the study population responding to this question ($n=425$). Most subjects ($n=91$) reported to suffer from allergic reactions to “other” sources, which were mainly described as cosmetic products or unknown elicitors. Other self-reported allergies were described for grass pollen ($n=46$), tree pollen ($n=23$) and mites ($n=19$). Self-reported allergies significantly correlated ($p < 0.05$) with sensitization, even though the percentage of positive molecule-based diagnosis within this group was only 59.3%.

Fisher’s exact tests were performed in 394 participants with diagnosed and self-reported allergies (Fig 6). IgE values correlated well with all reported diagnosed allergies while self-reported allergies correlated with the presence of specific IgE to allergens from mites, grass pollen and cat. No explicit statement can be made for the residual allergies due to a low prevalence. The number of participants reporting symptoms but not showing a sensitization to the respective source in the allergen microarray was generally low ($n=27$ and $n=34$ for diagnosed and self-reported, respectively). IgE reactivity to specific sources without allergic symptoms was 51.5% (grass pollen), 56.1% (cat hair), 71.6% (mites) and 76.6% (tree pollen). For insect venom, dog hair and weed pollen, high levels ($>90\%$) of asymptomatic individuals were noted.

DISCUSSION

This study analyzed IgE sensitization profiles of non-selected pupils from the region of Salzburg, Austria by means of the ImmunoCAP ISAC[®]. Sensitization data was additionally linked to reports on existing allergic diseases. Epidemiologic studies, like the one at hand, investigating a large number of allergens of diverse sources, are important to determine specific allergy elicitors in distinct geographic regions (6). A response rate of 76% of pupils from different school types allowed the analysis of an

unselected study population. Using the detection of 112 allergen molecules, we found an overall atopy rate of 53.5% in our adolescent study cohort. When, as suggested by Bousquet *et al.* (20), only 7 allergen sources would have been considered for this epidemiological study, the sensitization frequency would have decreased to 46.9%. This translates to a false-negative detection rate of more than 10% of atopic individuals. Therefore, a broad spectrum of allergens exploited for diagnostics considerably improves data quality. Prevalence rates similar to our study were also found in an adult population of the United States of America (44.6% and 54.3%) (21, 22) and for Taiwanese children aged 4-18 years (57.3%) (23). A geographically comparable cohort of 3-17 year-old children in Germany showed an atopy prevalence of 40.2% (24). On average, the participants from our study reacted to 8.6 single allergens, which is confirming the theory by Aalberse that once IgE is developed, individuals are predisposed to develop more IgE against other allergens (25). Our cohort reflects a population right at the beginning of a persistent allergic sensitization in adulthood. Therefore, we expect this population to progress towards more diverse and higher levels of allergen sensitizations following the epidemic trend of allergic diseases (26, 27).

In our cohort, we found a high prevalence of pollen sensitizations, with grass being the dominant antigenic source. Among pollen-sensitized subjects, 85% were reactive to grass pollen allergens which corresponds well with 81% prevalence among pollen-allergic patients from the Czech Republic, 200 km northeast of our study area (15). Analogous to previous studies on pollen-allergic patients, sensitization in our epidemiologic cohort was also mainly driven by group I grass allergens (15, 28). Except for 6 individuals, Phl p 5 reactivity was exclusively observed in Phl p 1-positive subjects, supporting the Phl p 1 initiator concept (29), but suggests inclusion of Phl p 5 for diagnosis. Interestingly, 23 individuals showed IgE reactivity to Cyn d 1 in the absence of Phl p 1 sensitization. Although Cyn d 1 is present as natural molecule, only 4/23 scored positive for the glycan marker MUXF3. The majority of Cyn d 1+/Phl p 1- subjects also reacted to Phl p 4, Pla a 2 or Jug r 2 and generally concomitant reactivity was frequently observed suggesting involvement of CCD epitopes. However, MUXF3 seems to be more limited in glycan diversity compared to those allergens from

natural sources. Determination of CCD is relevant for discrimination of clinical relevance in allergies, but requires additional markers aside from MUXF3 (30).

Sensitization to tree pollen allergens was mainly caused by Bet v 1 from birch pollen, which is typical for our region, but was less prevalent (40.2% vs 54.2%) compared to the patients' cohort from Panzner *et al.* (15). Notably, 24.4% were reactive to Ole e 1, which is considered a diagnostic marker for ash pollen sensitization in olive-free areas (31). Thus, ash sensitization seems to be more common than expected in our area and significantly higher compared to the Czech cohort with 9.3% (15). On the other hand, allergens from cypress and juniper pollen seem to play a neglectable role in Central Europe (32). English plantain was recently suggested as relevant source of pollinosis (33, 34). This finding was further corroborated by 24.9% sensitization prevalence to Pla l 1 among pollen-sensitized individuals in our study. Sensitization profiles of Pla l 1 and other Ole e 1-like family members were only partially overlapping, supporting recent results on limited IgE cross-reactivity of family members (35).

Sensitization to class 1 food allergens of walnut (n=42), shrimp (n=13), egg (n=7) and kiwi (n=6) was detected, while fish or cow's milk only play a negligible role. None of the participants reacted to the genuine peanut allergens Ara h 2/6, while reactivity to Ara h 8 occurred in 40 of the 85 PR-10-sensitized subjects (36). We therefore conclude that our region is not (yet) confronted with seed storage related peanut allergy triggering severe clinical reactions as observed in US or UK (37, 38). In general, IgE reactivity to food highly correlated with PR-10 reactivity and Bet v 1 represented the main driver of this pollen-food cross-reactivity (39). An almost hierarchical order of PR-10 sensitization is observed, showing the highest prevalence and IgE levels for Bet v 1 and the lowest for Act d 8 from kiwi.

We found significant differences in IgE levels when comparing pollen exclusive and multi-sensitized individuals. This might be due to the spread from major allergens towards minor ones (e. g. profilin) in a more "evolved" disease scenario (29, 39). Polysensitization was recently shown to affect the

probability of symptom development to furry animals (11), and was associated with asthma development in another study (40). A spreading phenomenon was also observed for the minor allergen Der p 10 from mites, which exclusively coincided with Der p 1/2 reactivity (41). For insect venom allergens, a higher reactivity to Api m 1 in the multi-sensitized group was found. Thus, a sensitization to Api m 1 seems to be more influenced by an already existing allergic/sensitized milieu, whereas sensitization to Ves v 5 is rather independent from other sensitizations. Of special interest is a weak but significant negative correlation of Ves v 5 with grass and mite allergens as well as a negative correlation between allergenic molecules from grasses and mites. This indicates that mite and wasp sensitization seem to be rather independent from other sources and interestingly, and IgE levels to Der p 1/2 were not elevated in multi-sensitized individuals.

Using a questionnaire, 55.5% of all subjects in our cohort reported to suffer from allergies. A Swedish study identified 42.3% subjects based on self-reported symptoms by questionnaires (42). The latter study investigated a mostly adult cohort (14-74 years) in a completely rural environment, which might relate to the lower incidence. A health survey of 1,099 adults from 2006 carried out all over Austria reported allergies in 20.8% of men and 23.1% of women (7).

While diagnosed allergies correlated with sensitization in 92% of cases, only 59% of self-reported allergics had allergen-specific IgE. Differences between the perception of allergic symptoms and the detection of IgE antibodies most likely reflect an uncertainty in the definition of allergic symptoms. Hence, self-reported symptoms might include non-IgE mediated reactions of the gastro-intestinal tract (including intolerances), dermatological reactions or non-allergic rhinitis (39). Similarly, the MAAS study also revealed 20% of children reporting allergic symptoms without IgE sensitization (43). In general, we found a very good correlation between IgE sensitization and positive diagnosis of specific sources, *i. e.* grass pollen and house dust mites. However, also self-reported allergies to grass pollen, house dust mite and cat hair correlated well with IgE reactivity. Although doctors' data are more robust, self-reported allergies are nevertheless valuable as those numbers would otherwise be missed in epidemiologic studies due to a reluctance of some patients to see a medical doctor.

This is the first epidemiologic study investigating IgE sensitization to single allergens in an unselected, adolescent population in the region of Salzburg, Austria. We found that 53.5% of the study cohort was sensitized displaying specific IgE to at least one allergen and hence is at an increased risk to develop an allergy (8). The unbiased study cohort gave us an important overview on the relevance of allergic sensitization at the molecular level and which allergens are necessary for inclusion in routine diagnosis in our area, e.g. Pla l 1 or Ole e 1. Such cohort studies provide a deep insight into IgE sensitization profiles, enable the monitoring of populations over time and can reveal prognostic molecules before onset of the disease (29). Since allergy represents a chronic disease with a tremendous number of people affected, the identification of valuable biomarkers for use in precision medicine is of utmost importance (44).

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AUTHORS' CONTRIBUTION

GG, MH, TH and JZ conceived the study

TS, EK, SM, LL, and GJO performed experiments

TS, ACB and RL analyzed data and calculated statistics

TS and GG wrote the manuscript

CONFLICT OF INTEREST

GG received lecture fees from Thermo Fisher Scientific. The rest of the authors declare no conflict of interest.

FIGURES

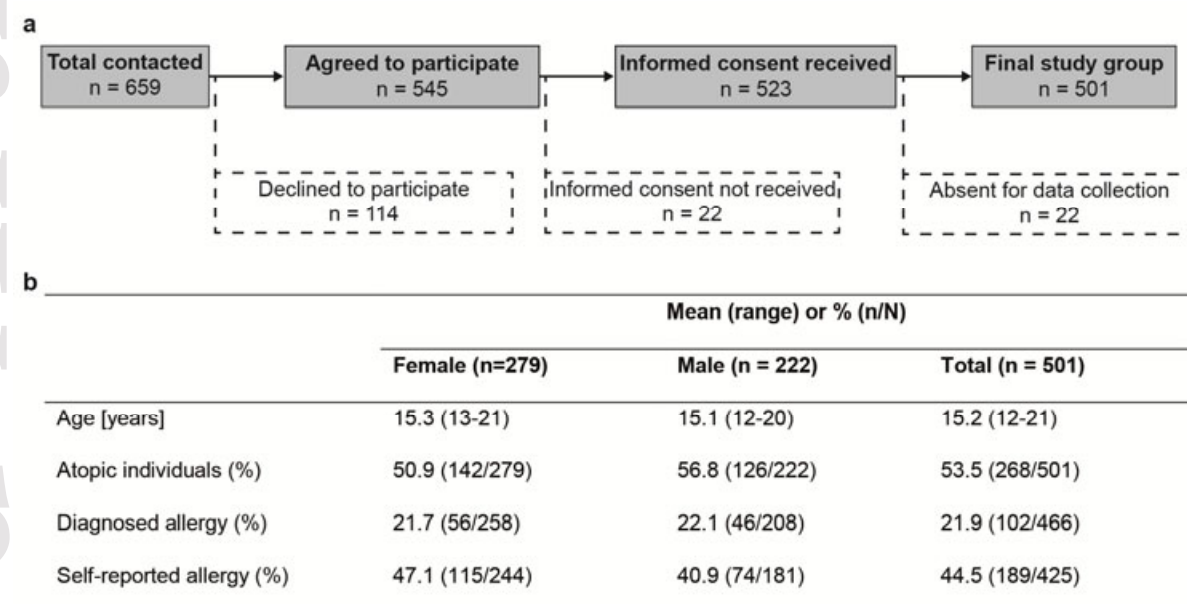


Fig.1. Participants' recruitment and table. **a**, Participation process in the study. Pupils were contacted in interested schools in the district of Salzburg, Austria. **b**, Demographic data of the study population. Values are given as mean with range or as percentage with n of N.

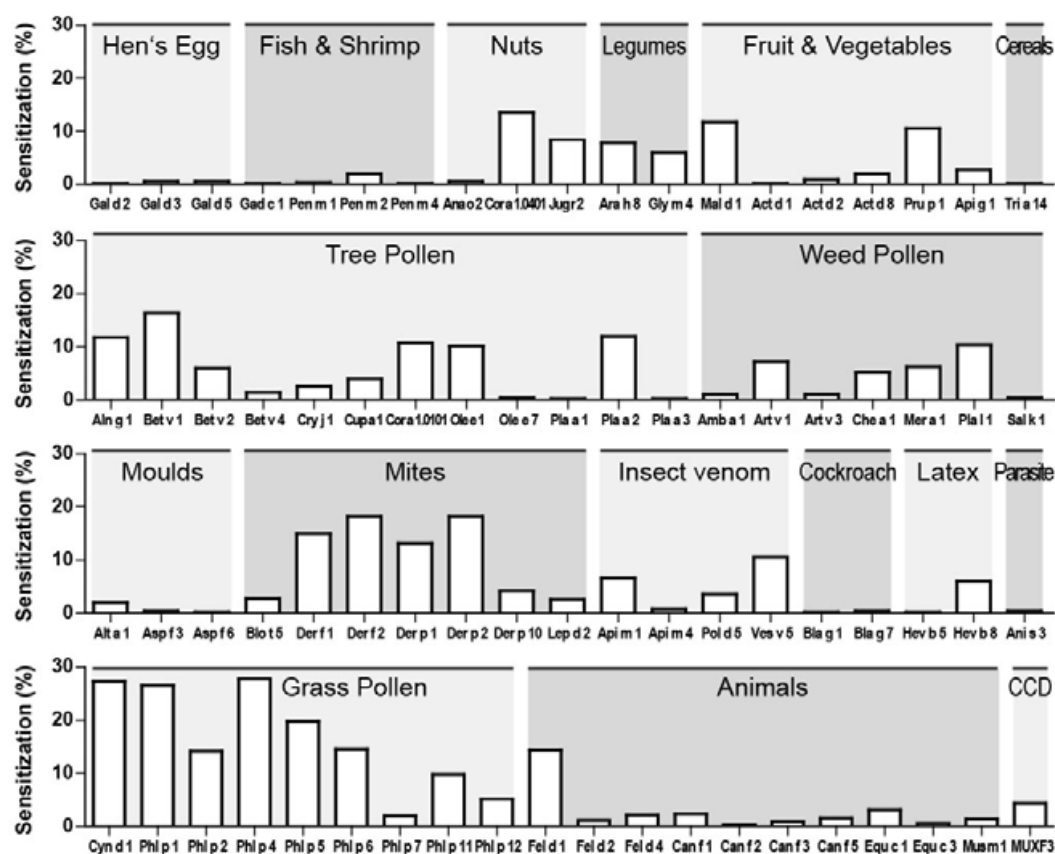
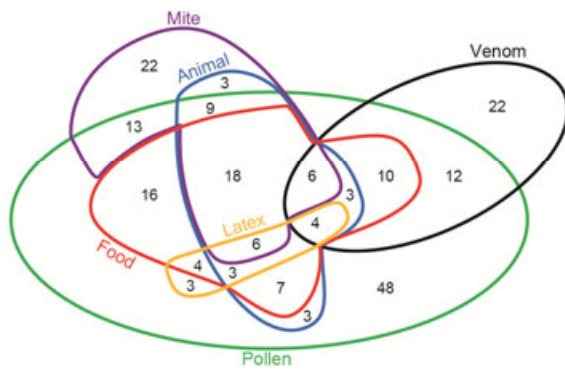
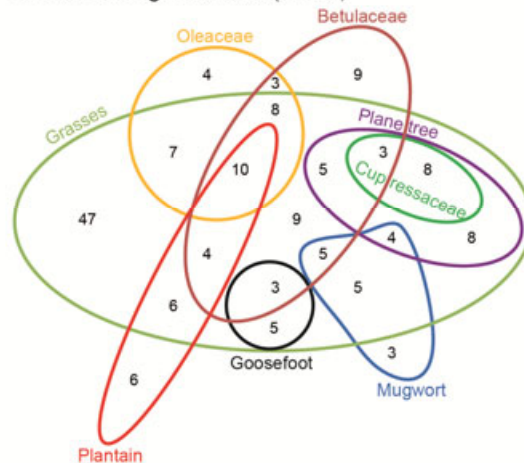


Fig. 2. Sensitization prevalence to molecules of different allergen sources. Seventy-six allergens and the CCD marker MUXF3 were detected positively ($ISU \geq 0.3$) at least once in the set of 112 components. Molecules are grouped by allergen source.

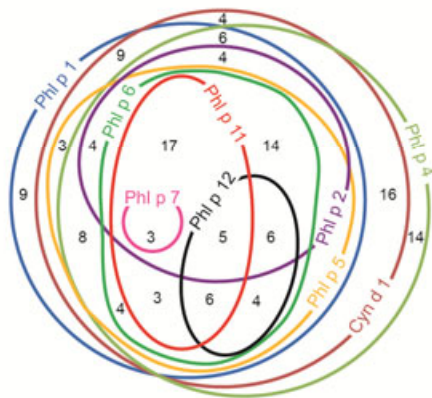
a All allergen sources (n = 268)



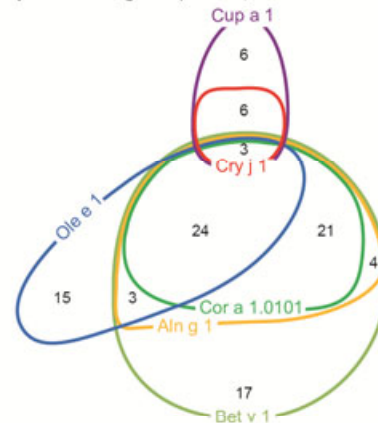
b Pollen allergen sources (n = 209)



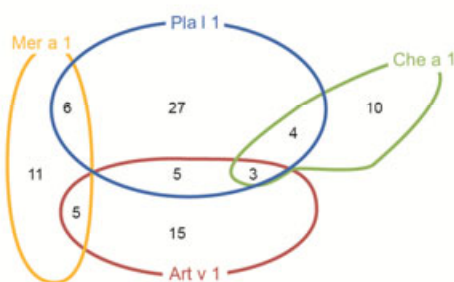
c Grass pollen allergens (n = 178)



d Tree pollen allergens (n = 142)



e Weed pollen allergens (n = 103)



f Ole e 1-like allergens (n = 106)

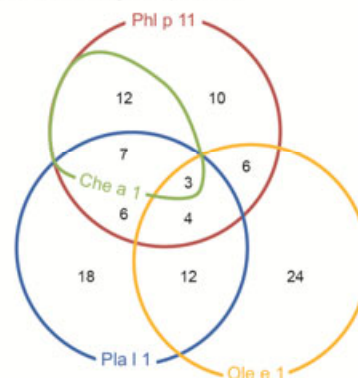
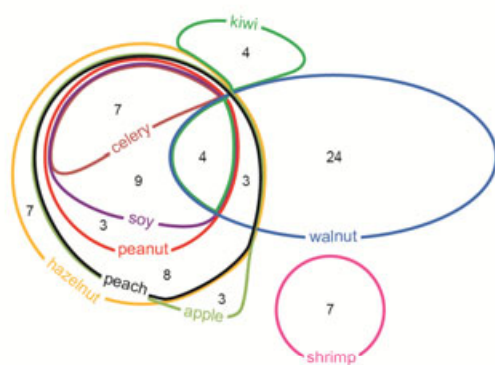
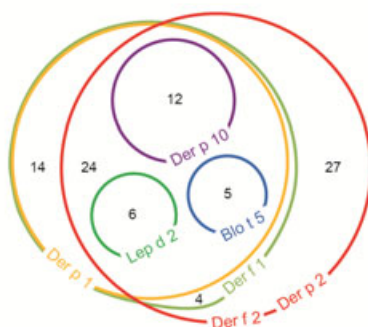


Fig 3. Set diagrams showing the IgE sensitization profile of the study population. **a**, All allergen sources; **b**, pollen allergens grouped by source; **c**, grass pollen allergens; **d**, tree pollen allergens; **e**, weed pollen allergens; **f**, Ole e 1-like allergens. Only groups containing 3 or more positive IgE samples are depicted; circle size approximately represents number of subjects

a Food allergens (n = 113)



b Mite allergens (n = 113)



c Insect venom allergens (n = 82)

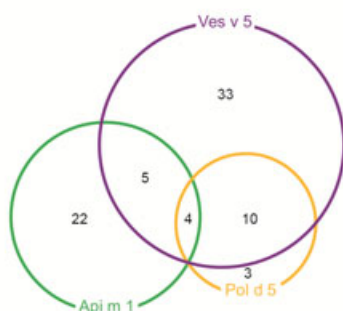


Fig 4. Set diagrams showing IgE sensitization profiles to specific allergen sources. **a**, Food allergens; **b**, mite allergens; **c**, insect venom allergens. Only groups containing 3 or more positive IgE samples are depicted; circle size approximately represents number of subjects.

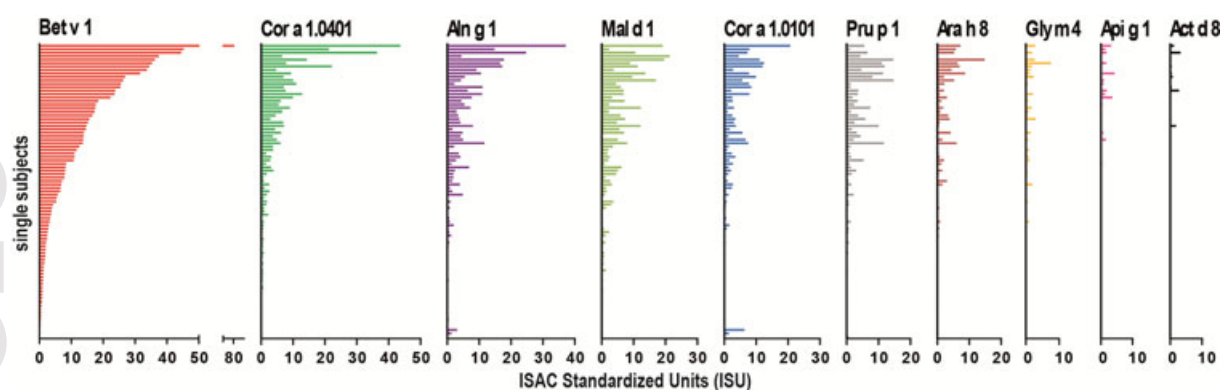


Fig 5. IgE sensitization to PR-10 allergen molecules. Horizontal bars represent PR-10-sensitized single subjects; sorted by ISU value to Bet v 1.

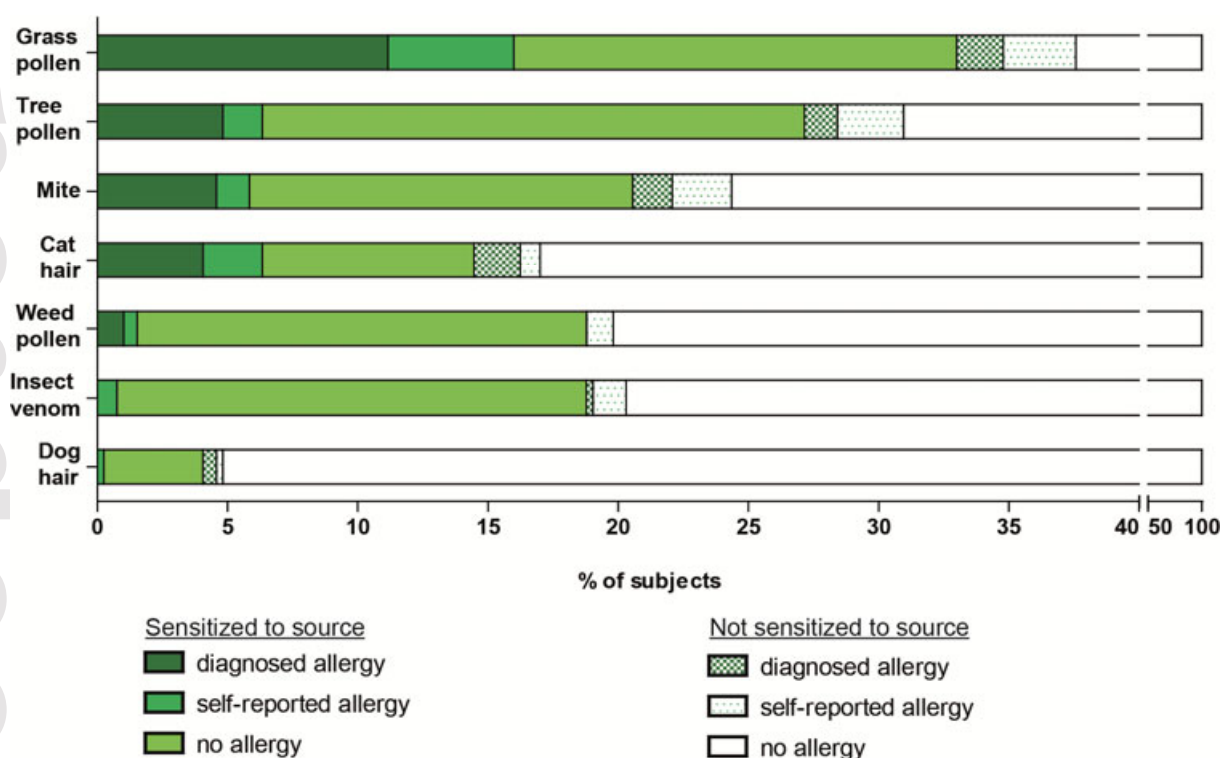


Fig. 6. IgE sensitization and allergies. Bars represent subjects sensitized to the respective allergen source in green shades; bars in white shades represent non-sensitized subjects. Only subjects that completed questions on diagnosed and self-reported allergies are shown (n=394).

REFERENCES

1. Bousquet J, Anto JM, Bachert C, Bousquet PJ, Colombo P, Cramer R, et al. Factors responsible for differences between asymptomatic subjects and patients presenting an IgE sensitization to allergens. A GA2LEN project. *Allergy* 2006;**61**(6):671-680.
2. Schernhammer ES, Vutuc C, Waldhor T, Haidinger G. Time trends of the prevalence of asthma and allergic disease in Austrian children. *Pediatr Allergy Immunol* 2008;**19**(2):125-131.
3. Wohrl S, Radon K, Ring J, Moritz K, Akdis C, Burney P, et al. [GA2LEN (Global Allergy and Asthma European Network), the perspective of the German speaking centers]. *Wien Klin Wochenschr* 2009;**121**(17-18):589-597.
4. Plebani M. Component-resolved diagnostics: laboratory results are not enough. *Clin Chem Lab Med* 2013;**51**(10):1887-1888.
5. Akdis CA, Agache I, editors. Global Atlas of Allergy: European Academy of Allergy and Clinical Immunology; 2014.
6. Canonica GW, Ansotegui IJ, Pawankar R, Schmid-Grendelmeier P, van Hage M, Baena-Cagnani CE, et al. A WAO - ARIA - GA(2)LEN consensus document on molecular-based allergy diagnostics. *World Allergy Organ J* 2013;**6**(1):17.
7. Dorner T, Lawrence K, Rieder A, Kunze M. Epidemiology of allergies in Austria. Results of the first Austrian allergy report. *Wien Med Wochenschr* 2007;**157**(11-12):235-242.
8. Olivieri M, Heinrich J, Schlunssen V, Anto JM, Forsberg B, Janson C, et al. The risk of respiratory symptoms on allergen exposure increases with increasing specific IgE levels. *Allergy* 2016.
9. Matricardi PM, Kleine-Tebbe J, Hoffmann HJ, Valenta R, Hilger C, Hofmaier S, et al. EAACI Molecular Allergology User's Guide. *Pediatr Allergy Immunol* 2016;**27 Suppl 23**:1-250.
10. Kleine-Tebbe J. Big-time sensitization rates in young Germans: big numbers--big risks--big confusion? *Int Arch Allergy Immunol* 2014;**163**(1):3-4.
11. Asarnoj A, Hamsten C, Waden K, Lupinek C, Andersson N, Kull I, et al. Sensitization to cat and dog allergen molecules in childhood and prediction of symptoms of cat and dog allergy in adolescence: A BAMSE/MeDALL study. *J Allergy Clin Immunol* 2016;**137**(3):813-821 e817.
12. Stringari G, Tripodi S, Caffarelli C, Dondi A, Asero R, Di Rienzo Businco A, et al. The effect of component-resolved diagnosis on specific immunotherapy prescription in children with hay fever. *J Allergy Clin Immunol* 2014;**134**(1):75-81.
13. Ferrer M, Sanz ML, Sastre J, Bartra J, del Cuvillo A, Montoro J, et al. Molecular diagnosis in allergology: application of the microarray technique. *J Investig Allergol Clin Immunol* 2009;**19 Suppl 1**:19-24.
14. Martinez-Aranguren R, Lizaso MT, Goikoetxea MJ, Garcia BE, Cabrera-Freitag P, Trellez O, et al. Is the determination of specific IgE against components using ISAC 112 a reproducible technique? *PLoS One* 2014;**9**(2):e88394.
15. Panzner P, Vachova M, Vitovcova P, Brodska P, Vlas T. A comprehensive analysis of middle-European molecular sensitization profiles to pollen allergens. *Int Arch Allergy Immunol* 2014;**164**(1):74-82.
16. Wahn U. What drives the allergic march? *Allergy* 2000;**55**(7):591-599.
17. von Elm E, Altman DG, Egger M, Pocock SJ, Gotsche PC, Vandenbroucke JP, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet* 2007;**370**(9596):1453-1457.
18. R Core Team. R: A Language and Environment for Statistical Computing. In. Vienna, Austria: R Foundation for Statistical Computing; 2016.
19. Mukaka MM. Statistics corner: A guide to appropriate use of correlation coefficient in medical research. *Malawi Med J* 2012;**24**(3):69-71.
20. Bousquet PJ, Hooper R, Kogevinas M, Jarvis D, Burney P. Number of allergens to be tested to assess allergenic sensitization in epidemiologic studies: results of the European Community Respiratory Health Survey I. *Clin Exp Allergy* 2007;**37**(5):780-787.

21. Salo PM, Arbes SJ, Jr., Jaramillo R, Calatroni A, Weir CH, Sever ML, et al. Prevalence of allergic sensitization in the United States: results from the National Health and Nutrition Examination Survey (NHANES) 2005-2006. *J Allergy Clin Immunol* 2014;**134**(2):350-359.
22. Arbes SJ, Jr., Gergen PJ, Elliott L, Zeldin DC. Prevalences of positive skin test responses to 10 common allergens in the US population: results from the third National Health and Nutrition Examination Survey. *J Allergy Clin Immunol* 2005;**116**(2):377-383.
23. Yao TC, Ou LS, Yeh KW, Lee WI, Chen LC, Huang JL. Associations of age, gender, and BMI with prevalence of allergic diseases in children: PATCH study. *J Asthma* 2011;**48**(5):503-510.
24. Schmitz R, Ellert U, Kalcklosch M, Dahm S, Thamm M. Patterns of sensitization to inhalant and food allergens - findings from the German Health Interview and Examination Survey for Children and Adolescents. *Int Arch Allergy Immunol* 2013;**162**(3):263-270.
25. Aalberse RC. Science from a black box. *J Allergy Clin Immunol* 2012;**130**(4):902-903.
26. Matricardi P. The allergy epidemic. In: Akdis C, Agache I, editors. Global Atlas of Allergy. Berlin: European Academy of Allergy and Clinical Immunology; 2014. p. 112-114.
27. De Amici M, Ciprandi G. The Age Impact on Serum Total and Allergen-Specific IgE. *Allergy Asthma Immunol Res* 2013;**5**(3):170-174.
28. Sastre J, Rodriguez F, Campo P, Laffond E, Marin A, Alonso MD. Adverse reactions to immunotherapy are associated with different patterns of sensitization to grass allergens. *Allergy* 2015;**70**(5):598-600.
29. Hatzler L, Panetta V, Lau S, Wagner P, Bergmann RL, Illi S, et al. Molecular spreading and predictive value of preclinical IgE response to Phleum pratense in children with hay fever. *J Allergy Clin Immunol* 2012;**130**(4):894-901 e895.
30. Villalta D, Conte M, Asero R, Da Re M, Stella S, Martelli P. Isolated IgE reactivity to native walnut vicilin-like protein (nJug r 2) on ISAC microarray is due to cross-reactive carbohydrate epitopes. *Clin Chem Lab Med* 2013;**51**(10):1991-1995.
31. Imhof K, Probst E, Seifert B, Regenass S, Schmid-Grendelmeier P. Ash pollen allergy: reliable detection of sensitization on the basis of IgE to Ole e 1. *Allergy J Int* 2014;**23**(3):78-83.
32. Pichler U, Hauser M, Wolf M, Bernardi ML, Gadermaier G, Weiss R, et al. Pectate lyase pollen allergens: sensitization profiles and cross-reactivity pattern. *PLoS One* 2015;**10**(5):e0120038.
33. Gadermaier G, Eichhorn S, Vejvar E, Weirnböck L, Lang R, Briza P, et al. Plantago lanceolata: an important trigger of summer pollinosis with limited IgE cross-reactivity. *J Allergy Clin Immunol* 2014;**134**(2):472-475.
34. Couto M, Miranda M. Proposed GA2LEN standardized allergen battery: what about regional sensitization differences? *J Investig Allergol Clin Immunol* 2011;**21**(6):491-492.
35. Stemeseder T, Freier R, Wildner S, Fuchs JE, Briza P, Lang R, et al. Crystal structure of Pla I 1 reveals both structural similarity and allergenic divergence within the Ole e 1-like protein family. *J Allergy Clin Immunol* 2016;**provisional acceptance for publication**.
36. Ackerbauer D, Bublin M, Radauer C, Varga EM, Hafner C, Ebner C, et al. Component-resolved IgE profiles in Austrian patients with a convincing history of peanut allergy. *Int Arch Allergy Immunol* 2015;**166**(1):13-24.
37. Sicherer SH, Munoz-Furlong A, Godbold JH, Sampson HA. US prevalence of self-reported peanut, tree nut, and sesame allergy: 11-year follow-up. *J Allergy Clin Immunol* 2010;**125**(6):1322-1326.
38. Nicolaou N, Poorafshar M, Murray C, Simpson A, Winell H, Kerry G, et al. Allergy or tolerance in children sensitized to peanut: prevalence and differentiation using component-resolved diagnostics. *J Allergy Clin Immunol* 2010;**125**(1):191-197 e191-113.
39. Westman M, Lupinek C, Bousquet J, Andersson N, Pahr S, Baar A, et al. Early childhood IgE reactivity to pathogenesis-related class 10 proteins predicts allergic rhinitis in adolescence. *J Allergy Clin Immunol* 2014.
40. Toppila-Salmi S, Huhtala H, Karjalainen J, Renkonen R, Makela MJ, Wang DY, et al. Sensitization pattern affects the asthma risk in Finnish adult population. *Allergy* 2015;**70**(9):1112-1120.

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41. Thomas WR. Hierarchy and molecular properties of house dust mite allergens. *Allergol Int* 2015;**64**(4):304-311.
 42. Enroth S, Dahlbom I, Hansson T, Johansson A, Gyllensten U. Prevalence and sensitization of atopic allergy and coeliac disease in the Northern Sweden Population Health Study. *Int J Circumpolar Health* 2013;**72**.
 43. Simpson A, Lazic N, Belgrave DC, Johnson P, Bishop C, Mills C, et al. Patterns of IgE responses to multiple allergen components and clinical symptoms at age 11 years. *J Allergy Clin Immunol* 2015;**136**(5):1224-1231.
 44. Muraro A, Lemanske RF, Jr., Hellings PW, Akdis CA, Bieber T, Casale TB, et al. Precision medicine in patients with allergic diseases: Airway diseases and atopic dermatitis-PRACTALL document of the European Academy of Allergy and Clinical Immunology and the American Academy of Allergy, Asthma & Immunology. *J Allergy Clin Immunol* 2016;**137**(5):1347-1358.